This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.
+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.
+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.
+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

3 3433 05767598 9

ELEMENTS

OF

WATER BACTERIOLOGY

WITH SPECIAL REFERENCE TO

SANITARY WATER ANALYSIS.

BY

SAMUEL CATE PRESCOTT,

Assistant Professor of Industrial Biology,

AND

CHARLES-EDWARD AMORY WINSLOW,

Instructor in Sanitary Bacteriology,

IN THE

MASSACHUSETTS INSTITUTE OF TECHNOLOGY.

FIRST EDITION.

FIRST THOUSAND.

NEW YORK:

JOHN WILEY & SONS.

LONDON: CHAPMAN & HALL, LIMITED.

1904.

287035

Copyright, 1904,
BY
S. C. PRESCOTT
AND
C.-E. A. WINSLOW.

ROBERT DRUMMOND, PRINTER, NEW YORK.

DEDICATED

TO

William Thompson Sedgwick

BY TWO OF HIS PUPILS,

AS A TOKEN OF GRATEFUL AFFECTION.

PREFACE.

THE general awakening of the community to the importance of the arts of sanitation—accelerated by the rapid growth of cities and the new problems of urban life—demands new and accurate methods for the study of the microbic world. Bacteriology has long since ceased to be a subject of interest and importance to the medical profession merely, but has become intimately connected with the work of the chemist, the biologist, and the engineer. To the sanitary engineer and the public hygienist a knowledge of bacteriology is indispensable.

In the swift development of this science during the last ten years perhaps no branch of bacteriology has made more notable progress than that which relates to the sanitary examination of water. After a brief period of extravagant anticipation, and an equally unreasonable era of neglect and suspicion, the methods of the practical water bacteriologist have gradually made their way, until it is recognized that, on account of their delicacy, their directness, and their certainty, these methods now furnish the final criterion of the sanitary condition of a potable water.

TABLE OF CONTENTS.

CHAPTER I.

PAGE

THE BACTERIA IN NATURAL WATERS..... 3

CHAPTER II.

THE QUANTITATIVE BACTERIOLOGICAL EXAMINATION OF WATER 19

CHAPTER III.

THE INTERPRETATION OF THE QUANTITATIVE BACTERIOLOGICAL ANALYSIS..... 35

CHAPTER IV.

DETERMINATION OF THE NUMBER OF ORGANISMS DEVELOPING AT THE BODY TEMPERATURE..... 43

CHAPTER V.

THE ISOLATION OF SPECIFIC PATHOGENES FROM WATER..... 49

CHAPTER VI.

METHODS FOR THE ISOLATION OF THE COLON BACILLUS..... 58

CHAPTER VII.

SIGNIFICANCE OF THE PRESENCE OF B. COLI IN WATER..... 74

CHAPTER VIII.

PRESUMPTIVE TESTS FOR B. COLI..... 94

CHAPTER IX.

PAGE

OTHER INTESTINAL BACTERIA..... 99

CHAPTER X.

THE SIGNIFICANCE AND APPLICABILITY OF THE BACTERIOLOGICAL EXAMINATION..... 109

APPENDIX..... 119
REFERENCES..... 129
INDEX..... 143

ELEMENTS OF WATER BACTERIOLOGY

ELEMENTS OF WATER BACTERIOLOGY.

CHAPTER I.

THE BACTERIA IN NATURAL WATERS.

BACTERIA are the most numerous and the most widely distributed of living things. They are present not merely at the surface of the earth or in the bodies of water which partially cover it, as is the case with most other living things, but in the soil itself, and in the air above, and in the waters under the earth.

Probably no organisms are more sensitive to external conditions, and none respond more quickly to slight changes in their environment. Temperature, moisture, and oxygen are of importance in controlling their distribution; but the most significant factor is generally the amount of food supply. Bacteria and decomposing organic matter are always associated, and for this reason a brief consideration of the general relation of bacteria to their sources of food supply and to other forms of organic life must precede the study of their distribution in any special medium.

First, then, the bacteria, though possessing greater constructive power than any animal organism, lack the power of green plants to build up their own food from purely inorganic materials, and must live upon the products of the growth of higher forms. A few species which have become adapted to a parasitic or semi-parasitic mode of life occur on the surface of the normal plant or animal body or penetrate the deeper layers of diseased tissues, feeding upon the fluids of the body or on the extraneous material collected upon its surface. Even these bacteria, however, may generally be cultivated under saprophytic conditions, and the vast majority of other forms live as true saprophytes on dead organic matter wherever it may occur in nature, and particularly in that diffuse layer of decomposing plant and animal material which we call the humus, or surface layer of the soil. Wherever there is life, waste matter is constantly being produced, and this finds its way to the earth or to some body of water. The excretions of animals, the dead tissues and broken-down cells of both animals and plants, as well as the wastes of domestic and industrial life, all eventually find their way to the soil. In a majority of cases these substances are not of such chemical composition that they can be utilized at once by green plants as food, but it is first necessary that they go through a fermentation or transformation in which their chemical composition becomes greatly changed; and it is as the agents of this transformation that bacteria assume their greatest importance in the world of life.

We may take a comparatively simple excretory product, urea, as an example. Through the activity of an enzyme produced by certain bacteria this compound unites with two molecules of water and is converted into ammonium carbonate,

$$CO\begin{matrix}\diagup NH_2 \\ \diagdown NH_2\end{matrix} + 2H_2O = (NH_4)_2CO_3.$$

While green plants can derive their necessary nitrogen in part, at least, from ammonium compounds it is a well-established fact that this element may be obtained much more readily from nitrates, and it is, therefore, essential as a further step that some means be employed to oxidize the nitrogen. This process of oxidation is known as *nitrification*, and takes place in a succession of steps, the organic nitrogen being first converted to the form of ammonium salts, and these in turn to *nitrites* and *nitrates*, the oxygen used coming from the air. Several groups of organisms are instrumental in bringing about this conversion. It is generally assumed that one group attacks the ammonium compounds and changes them to nitrites, while another group completes the oxidation to nitrates. In the latter form nitrogen is readily taken up by green plants to be built up into the more complex albuminoid substances (organic nitrogen) through the constructive power of chlorophyll.

This never-ending cycle is illustrated in the accompanying figure, devised by Sedgwick (Sedgwick, 1889) to illustrate the transformations of organic nitrogen in

nature, the increasing size and closeness of the spiral on the left-hand side indicating the progressive complexity of organic matter as built up by the chlorophyll bodies of green plants in the sunlight, and the other half of the figure the reverse process carried out largely by the bacteria. In nature there are many short circuits, as, for instance, when dead organic matter is used as food for animals and built up into the living state again without ever being nitrified and acted upon by green plants; but the complete cycle of organic nitrogen is as indicated on the diagram.

We have dwelt thus at length upon the general relation between bacteria and organic decomposition because in this relation will be found the master key to the distribution of bacteria in water as well as in other natural habitats. It is true that certain peculiar forms may at times multiply in fairly pure waters; but in general large numbers of bacteria are found only in connection with the organic matter upon which they feed. Such organic matter is particularly abundant in the surface layer of the soil. Here the processes of nitrification proceed most rapidly. Here the bacteria are most abundant; and in other media their numbers vary according to the extent of contact with the living earth. Natural waters particularly group themselves from a bacteriological standpoint in three well-marked classes, according to their relation to the rich layers of bacterial growth upon the surface of the globe. There are first the atmospheric waters which have never been subject to contact with the earth; second,

the surface-waters immediately exposed to such contamination in streams and ponds; third, the ground-waters from which previous pollution has been more or less removed by filtration through the deeper layers of the soil.

Even rain and snow, the sources of our potable waters, are by no means free from germs, but contain them in numbers varying according to the amount of dust present

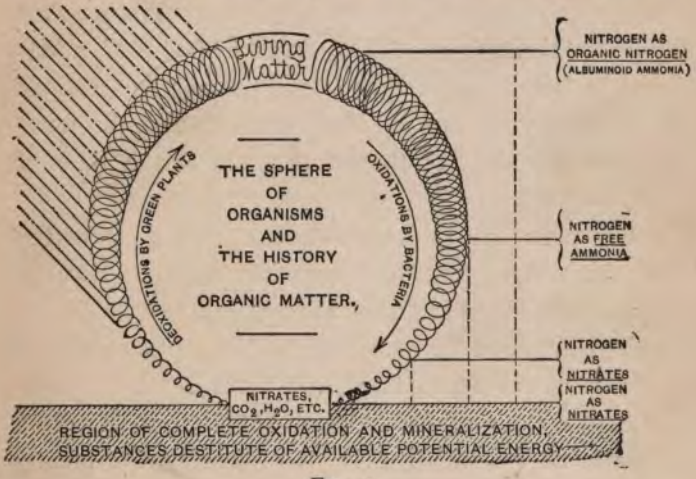

FIG. 1.

in the air at the time of the precipitation. After a long-continued storm the atmosphere is washed nearly free of bacteria, so that a considerable series of sterile plates may often be obtained by plating 1-c.c. samples. These results are in harmony with the observations of Tissandier (reported by Duclaux, 1897), who found that the dust in the air amounted to 23 mg. per cubic meter in Paris

and 4 mg. in the open country. After a rainfall these figures were reduced to 6 mg. and .25 mg., respectively.

With regard to what may be considered normal values for rain we have no very satisfactory figures. Those obtained by Miquel (Miquel, 1886) during the period 1883–1886, showing that rain contains on the average 4.3 bacteria per c.c. in the country (Montsouris) and 19 per c.c. in Paris, are probably lower than would be yielded by the present methods of examination. Snow shows rather higher numbers than rain. Janowski (Janowski, 1888) found in freshly fallen snow from 34 to 463 bacteria per c.c. of snow-water, and his results indicate that the number is independent of the temperature at the time of snowfall.

As soon as the rain-drop touches the surface of the earth its real bacterial contamination begins. Rivulets from ploughed land or roadways may often contain several hundred thousand bacteria to the cubic centimeter; and furthermore the amounts of organic and mineral matters which serve as food materials, and thus become a factor in later multiplication of organisms, are greatly increased.

In the larger streams several conditions combine to make the bacterial numbers lower. Ground-water containing little microbic life enters as a diluting factor from below. The larger particles of organic matter are removed from the flowing water by sedimentation; many earth bacteria, for which water is an unfavorable medium, gradually perish; and in general a new condition of equi-

librium tends to be established. A good river-water under favorable conditions should thus contain only a few hundred bacteria. Heavy rains which introduce wash from the surrounding watershed may, however, at any time upset this condition of equilibrium and surface-waters are apt to show sudden fluctuations in their bacterial content. Particularly in the spring and fall high numbers manifest themselves, and seasonal variations arise, such as are well shown in the appended table.

NUMBER OF BACTERIA PER CUBIC CENTIMETER IN CERTAIN SURFACE-WATERS.

Water.	Yr.	Observer.	Jan.	Feb.	March.	April.	May.	June
Boston tap water.....	1892–1895	Whipple*	135	211	102	52	53	86
Merrimac River.	1901	Clark †	5,600	2,500	8,900	1,400	1,400	3000
Thames River...	1888	Frankland‡	92,000	40,000	66,000	13,000	1,900	3500
River Ourcq..	1887–1890	Miquel §	143,370	63,720	47,780	22,660	29,340	7340

Water.	Yr.	Observer.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Boston tap water.....	1892–1895	Whipple*	73	81	86	55	56	52
Merrimac River.	1901	Clark†	1760	1580	1760	1,900	1,300	5,100
Thames River...	1888	Frankland‡	1070	3000	1740	1,130	11,700	10,600
River Ourcq..	1887–1890	Miquel §	7730	8520	8070	12,560	135,700	153,200

* Whipple, 1896. † Massachusetts State Board of Health, 1902.
‡ Frankland, 1894. § Miquel, 1891.

Two factors influence this seasonal distribution of bacteria. First, during the summer months the water flowing in open rivers is largely derived from springs and subterranean sources, while during the autumn and spring months, there is a much greater proportion of "run-off" water contaminated by contact with the sur-

face of the earth. In the second place, during these rainy seasons the amount of dissolved organic matter is far greater than in summer, thus making the food-supply of the bacteria more abundant.

In standing waters all the tendencies which make for the reduction of bacteria are intensified, and ponds and lakes often give numbers under a hundred. The student will find numerous analyses of natural waters in Frankland's classic work (Frankland, 1894). He notes, for example, that the Lake of Lucerne contained 8 to 51 bacteria per c.c., Loch Katrine 74, and the Loch of Lintralthen an average of 170. The water of Lake Champlain examined by one of us (S. C. P.) in 1896 contained on an average 82 bacteria per c.c. at a point more than two miles out from the city of Burlington. Certain surface water-supplies near Boston studied by Nibecker and one of us (Winslow and Nibecker, 1903) gave the following results:

City.	Number of Samples.	Average Number of Bacteria per cc.
Wakefield	7	59
Lynn	6	16
Plymouth	6	35
Cambridge	5	94
Salem	5	232
Medford	5	524
Taunton	4	13
Peabody	3	141

Russell found similar small numbers in sea-water at Naples (Russell, 1891) and Wood's Hole (Russell, 1892).

and in salt as in fresh water the amount of bacterial life decreases in general as one passes downward from the surface and outward from the shore.

The principal factors in the destruction of the bacteria in water during storage appear to be sedimentation, the activity of other micro-organisms, light, temperature, and food-supply, and perhaps more obscure conditions such as osmotic pressure.

The subsidence of bacteria either by virtue of their own specific gravity or as the result of becoming attached to particles of suspended matter is unquestionably partly, if not largely, responsible for changes in the number of bacteria in the upper layers of water at rest or in very sluggish streams. The results of numerous investigations by different workers seem to indicate that sedimentation takes place slowly, and that the difference in numbers between the top layer and the bottom layer of water in tall jars in laboratory experiments of only a few days' duration is very slight or quite within the limits of experimental error (Tiemann and Gärtner, 1889). Different species may, of course, be differently affected, (Scheurlen, 1891). It must be remembered, that in natural streams bacteria are to a great extent attached to larger solid particles upon which the action of gravity is much more important. Jordan (Jordan, 1900) is firmly of the opinion that in the lower part of the Illinois River, where there is a fall of but 30 feet in 225 miles, the influences summed up by the term sedimentation are sufficiently powerful to obviate the

necessity for summoning another cause "to explain the diminution in numbers of bacteria," and he further adds: "It is noteworthy that all the instances recorded in the literature where a marked bacterial purification has been observed are precisely those where the conditions have been most favorable for sedimentation."

Little is known as to the share of other organisms in hastening the decrease of bacteria in stored water. Doubtless predatory Protozoa play some part here, and certain bacteriologists have believed that the toxic waste products of some species of bacteria materially check the development of other forms. Horrocks (Horrocks, 1901), Garré (Garré, 1887), Zagari (Zagari, 1887), and Freudenreich (Freudenreich, 1888) have shown that an "antagonism" exists when bacteria are grown in artificial culture media such that the substratum which has supported the growth of one form may be rendered antiseptic to another. It is difficult, however, to believe that any poisons are produced of such enormous power as to cause this effect in a stream or a lake, and there is no evidence in support of such a view.

Temperature has a direct relation to bacterial life, and the number of parasitic bacteria at least may be quickly lessened by the action of cold. Sedgwick and one of us (Sedgwick and Winslow, 1902) have shown that of typhoid bacilli in ice or cool water over 40 per cent will perish in three hours and 98 per cent and upwards in two weeks.

Many investigations conducted since the pioneer researches of Downes and Blunt (Downes and Blunt, 1877) have confirmed the results reported by them, viz., direct sunlight is fatal to most bacteria in the vegetative state and even to spores if the exposure be sufficiently long, while diffused light is harmful in a lesser degree. Opinions vary as to the degree to which light is active in destroying the bacteria in natural water. Buchner (Buchner, 1893) found by experiment that the bactericidal power of light extends to a depth of about three meters before it becomes imperceptible. On the other hand, Procaccini (Procaccini, 1893) found that when sunlight was passed vertically through 60 cm. of drain-water the lower layers contained nearly as many bacteria after three hours' treatment as before the exposure. The middle and upper portions showed a great falling off in numbers, however.

But few studies have been made of the effect of light on bacteria in flowing water. Jordan (Jordan, 1900) has investigated several Illinois streams and arrived at the conclusion that in moderately turbid water, at least, the sun's rays are virtually without action. On the other hand, Rapp has observed a considerable reduction of the bacteria in the Isar at Pullach after the period of diurnal insolation, as shown by the table on the following page.

Although it is hard to estimate the exact importance of each factor, the general phenomena of the self-purification of streams are easy to comprehend. A small

EXAMINATIONS OF THE ISAR AT PULLACH (RAPP, 1903).

(A) CARRIED OUT SEPTEMBER 26, 1898, NO RAIN HAVING FALLEN FOR THREE WEEKS.

Temperature		Time of the Experiment.	Bacteria per cc.
of the Water.	of the Air.		
13.0° C.	8.8° C.	7.30 P.M.	146
12.1° C.	7.0° C.	9.30 P.M.	270
10.5° C.	6.2° C.	5.00 A.M.	370
10.2° C.	8.2° C.	8.00 A.M.	320
(B) CARRIED OUT NOVEMBER 28, 1898, NO RAIN HAVING FALLEN FOR SOME TIME.			
5.5° C.	3.0° C.	6.00 P.M.	266
5.5° C.	2.5° C.	8.00 P.M.	402
5.5° C.	2.0° C.	10.00 P.M.	482
5.0° C.	2.0° C.	3.00 A.M.	532
4.5° C.	2.5° C.	7.30 A.M.	400

brook immediately after the entrance of polluting material from the surface of the ground contains a large number of bacteria from a diversity of sources. Gradually those organisms adapted to life in the earth or in the bodies of plants and animals die out, and the forms for which water furnishes ideal conditions survive and multiply. It is no single agent which brings this about, but that complex of little-understood conditions which we call the environment. If any one thing is of prime importance it is probably the food-supply, for only certain bacteria are able to multiply in the presence of the small amount of organic matter present in ordinary potable waters. As Jordan (Jordan, 1900) has said: "In the causes connected with the insufficiency or unsuitability

of the food-supply is to be found, I believe, the main reason for the bacterial self-purification of streams."

In general we have seen then that surface-waters tend continually to decrease in bacterial content after their first period of contact with the humus layer of the soil. In that other portion of the meteoric water which penetrates below the surface of the earth to join the reservoir of ground-water, later to reappear as the flow of springs and wells, this diminution is still more marked since the filtering action of the earth removes not only most of the bacteria but much of their food material as well. Indeed many observers formerly believed that all ground-waters were nearly free from bacteria, because often no colonies appeared on plates counted after the ordinary short periods of time. If, however, a longer period of incubation be adopted considerable numbers may be obtained.

For convenience we may divide ground-waters into two groups, namely: first, springs and shallow open wells, and second, "tubular" (driven) or deep wells. This division is a convenient one because ordinary springs and wells form a group by themselves in respect to the possibility of aerial and surface contamination, their water often being fairly rich in bacterial life. Egger (Wolffhügel, 1886) examined 60 wells in Mainz and found that 17 of them contained over 200 bacteria to the cubic centimeter. Maschek (Maschek, 1887) found 36 wells out of 48 examined in Leitmeritz which had a bacterial content of over 500 per c.c. Fischer (Horrocks, 1901) reported 120 wells

in Kiel which gave over 500 bacteria per c.c. and only 51 with less than that number.

Several unpolluted springs and open wells were examined by Sedgwick and one of us (Sedgwick and Prescott, 1895) with the following result:

Spring	No. 1.	252,	255,	258	bacteria per	c.c.
"	" 2.	163,	149,	134	" "	"
"	" 3.	92,	98,	105	" "	"
"	" 4.	95,	101,	106	" "	"
"	" 5.	193,	213,	218	" "	"
"	" 6.	216,	208,	201	" "	"

OPEN WELLS.

No. 1.	509,	525	bacteria per	c.c.		
" 2.	248,	190	"	"	"	
" 3.	602,	560	"	"	"	
" 4.	335,	332	"	"	"	
" 5.	2084,	2063,	2287	bacteria per	c.c.	
" 6.	8905,	8905,	8640	"	"	"
" 7.	702,	910,	871	"	"	"
" 8.	720,	712,	763	"	"	"

It should be noted that the above results were obtained by incubating the plates for considerable periods of time. In the ordinary standard 48-hour period but very few bacteria develop from normal ground-waters. Thus in an examination of spring-waters made by the Massachusetts State Board of Health in 1900 (Massachusetts State Board of Health, 1901), of 37 springs which were practically unpolluted and had less than 10 parts per 100,000 excess of chlorine over the normal, 54 samples were examined and gave an average of 41 bacteria per c.c. Only 6 samples showed figures over 50. On the other hand, the analysis of the bottled samples of the same waters as sold after exposure to contamination in

bottling and the multiplication of the water bacteria gave numbers rising to 243,000, only 14 out of 50 samples being under 1000.

It now remains to consider the other great division of ground-waters, namely, deep, "driven," or "tubular" wells, which, if carefully constructed, should ordinarily be perfectly free from all surface-water contamination. The numbers of bacteria in such sources has been reported by but few investigators. A series of wells in and near Boston was found to give the following figures (Sedgwick and Prescott, 1895).

Well.	Depth, Feet.	Bacteria per c.c.
No. 1	193	269, 254
" 2	100	30
" 3	454	206, 214
" 4	254	150, 135
" 5	...	228
" 6	198	192, 193
Second sample		262, 258
" 7	213	139, 140
" 8	213	101, 106
Second sample		408, 416
" 9	377	48, 54
Second sample		158, 149
" 10	227	1240, 1376
" 11	130	440, 480
" 12	200	525
" 13	180	60, 57
" 14	750	38

Again it should be noted that the period of incubation for these samples was at least five days. Fifteen driven wells in the neighborhood of Boston examined in 1903 showed at the end of 48 hours an average of only 18 colonies per c.c.

It is plain that water absolutely free from bacteria is not ordinarily obtained from any source and that even deep wells contain quite appreciable numbers. The peculiar character of the organisms present in the latter case is manifested in many cases by the slow development at room temperature (no growth until the third day in some cases), the entire absence of liquefying colonies, and the abundance of chromogenic species.

CHAPTER II.

THE QUANTITATIVE BACTERIOLOGICAL EXAMINATION OF WATER.

THAT the customary methods for determining the number of bacteria do not reveal the total bacterial content, but only a very small fraction of it, becomes apparent when we consider the large number of organisms, nitrifying bacteria, cellulose-fermenting bacteria, strict anærobes, etc., which refuse to grow, or grow only very slowly in ordinary culture media, and which, therefore, escape our notice. On the one hand certain obligate parasites cannot thrive in the absence of the rich fluids of the animal body; on the other hand the prototrophic bacteria adapted to the task of wrenching energy from nitrates and ammonium compounds are unable to develop in the presence of so much organic matter. This is made clear by the use of special media like the Nährstoff Heyden agar (Hesse and Niedner, 1898), which are particularly adapted to the needs of these latter organisms; or by direct microscopic examination. Thus Gage and Phelps (Gage and Phelps,

---

Note. In this chapter the authors have closely followed the recommendations of the A. P. H. A., as given in the Appendix.

1902) showed that the numbers obtained by the ordinary procedure were only from 5 to 50 per cent of those obtained by the use of Heyden's Nährstoff agar. For practical sanitary purposes, however, our methods are fairly satisfactory. Within limits, it is of no great importance that one method allows the growth of more bacteria than another. When we are using the quantitative analysis as a measure of sewage pollution only two things are essential. First, media should be of standard composition, so that results obtained at different times and by different observers may be comparable. In this respect the work of G. W. Fuller, G. C. Whipple, and other members of the Committee on Standard Methods of the American Public Health Association has placed the art of quantitative water analysis in a state, very satisfactory, by contrast with the chaos which prevails in England and Germany. Secondly, it is desirable that the section of the total bacterial flora which we obtain should be thoroughly representative of that portion of it in which we are most interested—the group of the quickly growing, rich-food-loving sewage forms. In this respect our meat-gelatin-peptone appears to be unrivalled. The following table from Gage and Phelps's valuable paper shows clearly that the standard media bring out the difference between pure and polluted waters much more clearly than does the Nährstoff medium. To emphasize this difference with constancy is all that we require of a method for practical work.

TABLE SHOWING PERCENTAGES OF BACTERIA DEVELOPING ON REGULAR AGAR AND ON NÄHRSTOFF AGAR FOR DIFFERENT CLASSES OF WATERS. (GAGE AND PHELPS, 1902.)

Class of Water.	Days' Count.						
	2	3	4	5	6	7	8
REGULAR AGAR.							
Ground-water	0	5	6	6	6	6	6
Filtered water	6	7	7	7	7	7	7
Merrimac River	6	7	7	8	8	9	9
Filtered sewage	14	17	18	19	19	19	19
Sewage	34	44	46	46	46	46	46
NÄHRSTOFF AGAR.							
Ground-water	6	43	78	88	93	100	100
Filtered water	37	69	80	92	98	100	100
Merrimac River	29	78	93	97	97	99	100
Filtered sewage	26	65	93	95	97	99	100
Sewage	39	75	95	100	100	100	100

The procedure for the quantitative determination of bacteria in water consists, in brief, in mixing a definite amount of a suitably collected specimen of the water with a sterile solidifiable culture medium and allowing it to develop for a sufficiently long time to permit reproduction of the bacteria and the formation of visible colonies which may be counted. The process is divided naturally into four stages—sampling, plating, incubating, and counting—which will now be discussed.

Sampling.—All samples of water for bacteriological examination should be collected in clean, sterile bottles with wide mouths and glass stoppers, preferably of the flat

mushroom type. It is desirable that these bottles should have a capacity of at least 100 c.c.

They should be cleaned thoroughly before using, by treatment with sulphuric acid and potassium bichromate or with alkaline permanganate of potash followed by sulphuric acid, dried by draining, and sterilized by dry heat at 160° C. for at least one hour, or by steam at 115°–120° for fifteen minutes. If not to be used immediately the neck and stopper should be protected against dust or other contamination by wrapping with lead-foil. For transportation the bottle should be enclosed in a suitable case or box.

The greatest care must be taken that the fingers do not touch the inside of the neck of the bottle or the cone of the stopper, as the water thereby would become seriously contaminated and rendered unfit for examination. It is well known that bacteria are found abundantly upon the skin, and Winslow (Winslow, 1903) has shown that even B. coli is present upon the hands in a considerable number of cases.

In order to obtain a fair sample, great precautions must be taken, and these will vary with the different classes of waters to be examined and with local conditions. If a sample is to be taken from a tap, the water should be allowed to flow at least five minutes (if from a tap in regular use) or for a longer period in case the water has been standing in the house service system, since in the small pipes, changes in bacterial content are liable to occur,

certain species dying, and the sample, therefore, not being a fair average of the water in the mains.

If a sample is to be taken from a pump similar precautions are necessary. The pump should be in continuous operation for several minutes at least, and preferably for half an hour before the sample is taken, in order to avoid excessively high numbers due to the growth of bacteria within the well and pump, the bacterial condition of the water as it passes through the ground being what we wish to determine. Thus Heræus (Heræus, 1886) in a well-water which had been but little used during the preceding thirty-six hours found 5000 organisms per c.c.; when the well was emptied by continuous pumping a second sample, after an interval of half an hour, gave only 35. Maschek (Tiemann and Gärtner, 1889) obtained similar results shown in the following table:

EFFECT OF PUMPING ON THE BACTERIAL CONTENT OF WELL-WATER.

Well-water after continuous pumping for fifteen minutes	458
" " " " " many hours	140
" later	68
" after continuous pumping for fifteen minutes	578
" " " " " many hours	179
" later	73

After a proper interval of pumping the sample of a well-water may be collected from the pet-cock of the pump or from a near-by tap. With a hand-pump, as in sampling domestic shallow wells, the water is, of course, pumped directly into the sample bottle. The difficulties in securing an average sample from this latter source are

often great, since if the flooring about the pump is not tight, as is usually the case, continued pumping may wash in an unusual amount of surface pollution.

In sampling surface-waters, the greatest precautions must be observed to prevent contamination from the fingers. In still waters the fairest sample is one taken from several inches down, as the surface itself is likely to have numerous dust particles floating upon it. The method most frequently recommended is to plunge the bottle beneath the surface to a depth of a foot or so, then remove the stopper and allow the bottle to fill.

Another method which is comparatively free from objection and which has been employed by the writers is to remove the stopper first and then, holding the bottle by the base, plunge it mouth downward into the water, turning it at the desired depth so as to replace the enclosed air by the water. Whenever any current exists the mouth of the bottle should be directed against it in order to carry away any bacteria from the fingers. If there is no current, a similar effect can be produced by turning the bottle under water and giving it a quick forward motion. In rapidly flowing streams it is only necessary to hold the bottle at the surface with the mouth pointed up-stream.

For taking samples of water at greater depths, a number of devices have been employed, all of which are fairly satisfactory. The essentials are, first, a weight to carry the bottle down to the desired depth, and, second, a device

for removing the stopper when that depth is reached. The student will find one good form of apparatus described in Abbott's "Principles of Bacteriology" (Abbott, 1899); an admirable one was devised by Hill and Ellms (Hill and Ellms, 1898). Miquel and Cambier (Miquel and Cambier, 1902) and other authors recommend the use of a sealed glass bulb with a capillary tube which can be broken off at any desired moment.

As soon as a sample of water is collected its conditions of equilibrium are upset and a change in the bacterial content begins. Even in the purest spring-waters which contain but few bacteria when collected, and in which the amount of organic matter is infinitesimal, enormous numbers will be found after storage under laboratory conditions for a few days or even a few hours. In some cases the rise in numbers is gradual, in others very rapid. The Franklands (Frankland, 1894) record the case of a deep-well water in which the bacteria increased from 7 to 495,000 in three days. Miquel (Miquel, 1891), from his researches, arrived at the conclusion that in surface-waters the rise is less rapid than in waters from deep wells or springs, and that in the latter case the decrease, after reaching a maximum, is likewise rapid and steady. Just how far protection from light, increase in temperature, and a destruction of higher micro-organisms is responsible for the increase, and to what extent an exhaustion of food-supply or the formation of toxic waste products causes the succeeding decrease, we are not aware; but the facts are well established.

Whipple has exhaustively studied the details of this multiplication of bacteria in stored waters and has shown in the table given below that there is first a slight reduction in the number present, lasting perhaps for six hours, followed by the great increase noted by earlier observers. It is probable that there is a constant increase of the typical water bacilli, overbalanced at first by a reduction in other forms, for which this is an unsuitable environment.

BACTERIAL CHANGES IN WATER DURING STORAGE.

(Whipple, 1901.)

Sample.	Initial Temperature.	Temp. of Incubation of Sample.	Number of Bacteria per c.c.				
			Initial.	After 3 Hours.	After 6 Hours.	After 24 Hours.	After 48 Hours.
	C.	C.					
A	7.6°	17.0°	260	215	230	900	27,000
B	7.6°	17.0°	260	245	255	720	10 850
C	7.6°	12.5°	260	270	231	600	2,790
D	7.6°	12.5°	260	270	245	710	1,800
E	7.6°	2.4°	260	243	210	675	1,980
F	7.6°	2.4°	260	235	270	560	1,980
G	11.0°	12.8°	77	55	58	101	10 250
H	11.0°	12.8°	77	53	74	87	2.175
I	11.0°	23.6°	77	51	52	11,000	41,400
J	6.7°	20.0°	430	375	245	.....	385 000*
K	6.7°	20.0°	430	345	405	.....	750,000*
L	23.2°	23.0°	510	340	230	8,000	20,000
M	23.2°	2.5°	525	300	220	380	2,200

* 0.0005 per cent peptone added to the water.

Wolffhügel and Riedel (Wolffhügel and Riedel, 1886) noted the dependence of this multiplication on the amount of the air-supply, vessels closed with rubber stoppers showing lower numbers than those plugged with cotton. Similarly, Whipple found that the multiplication of bacteria

was much greater when the bottles were only half full than when they were filled completely; and also, as shown in the following very striking table, that the size of the bottle markedly influenced the growth.

EFFECT OF SIZE OF VESSEL UPON THE MULTIPLICATION OF WATER BACTERIA DURING STORAGE.

(Whipple, 1901.)

Sample	Bottle.	Temp. of Incubation.	Number of Bacteria per c.c.					
			Initial.*	After 3 hrs.	After 6 hrs.	After 12 hrs.	After 24 hrs.	After 48 hrs.
		C.						
A	1-gallon	13°	77	63	65	47	42	175
B	2-quart	13°	77	59	63	60	45	690
C	1-quart	13°	77	63	63	47	46	325
D	1-pint	13°	77	57	61	36	38	630
E	2-ounce	13°	77	55	58	47	101	10,250
F	1-gallon	24°	77	81	97	275	290	300
G	2-quart	24°	77	92	59	62	180	250
H	1-quart	24°	77	84	77	46	340	900
I	1-pint	24°	77	51	46	100	2,950	7,020
J	2-ounce	24°	77	51	52	145	11,000	41,400

* Average of five plates.

These results and those of other observers make it obvious that samples must be examined shortly after collection and that they must be kept cool during their storage. If fairly pure waters are placed upon ice and kept between 0° and 10°, they will show no material increase in twelve hours. With polluted water, however, another danger is here introduced. Samples of such water when packed in ice show a marked decrease due to the large number of sensitive intestinal bacteria present. Jordan (Jordan, 1900) found that three samples of river-water

packed in ice for forty-eight hours fell off from 535,000 to 54,500; from 412,000 to 50,500, and from 329,000 to 73,000, respectively. It is, therefore, necessary to adhere strictly to the recommendations of the A. P. H. A. Committee that the interval between sampling and examination should not exceed twelve hours in the case of relatively pure waters, six hours in the case of relatively impure waters, and one hour in the case of sewage.

Plating.—The bottle containing the sample of water is first shaken at least twenty-five times in order to get an equal distribution of the bacteria. If the number of bacteria present is probably not greater than 200, 1 c.c. is then withdrawn with a sterile 1 c.c. pipette and delivered into a sterile Petri dish of 10 cm. diameter. To this is added 5 c.c. of standard 10 per cent gelatin at a temperature of about 30° C. or standard agar (7 c.c.) at 40°–42° C Should the number of bacteria per c.c. probably exceed 200, dilution is necessary. This is best accomplished by adding 1 c.c. of the water in question to 9, 99, or 999, etc., c.c. of sterile tap water according to the amount of dilution required. The diluted sample is then shaken thoroughly and 1 c.c. taken for enumeration. In order to determine the number of bacteria originally present it is only necessary to multiply by the factor 10, 100, or 1000, etc.

When a sample of water from an unknown source is to be examined it is generally desirable to make a series of plates at each of the above dilutions, selecting those which give nearest to 200 colonies on the plates after incubation as the ones on which to rely for the count. A much

smaller number will not give average figures, and if more than 200 colonies are present on a plate many bacteria will be checked by the waste products of those which first develop and the count obtained will be too low. After the addition of the diluted sample and the nutrient medium their thorough mixture in an even layer on the bottom of the plate is obtained by careful tipping and rotation.

It was formerly customary to mix the water with the gelatin in the tube before pouring into the plate, but this method is objectionable because there is always a small residue of medium remaining in the tube which will retain varying numbers of bacteria and thus interfere with the accuracy of the count. Before pouring the medium into the plate the mouth of the tube should be flamed to remove any possibility of contamination.

The exact composition of the medium used is, of course, of prime importance in controlling the number of bacteria which will develop. The reaction of the medium was found as early as 1891 to be important, for Reinsch (Reinsch, 1891) showed in that year that the addition of one one-hundredth of a gram of sodium carbonate to the liter increased sixfold the number of bacteria developing. Fuller (Fuller, 1895) and Sedgwick and one of ourselves (Sedgwick and Prescott, 1895), working independently, established the fact that an optimum reaction existed for most water bacteria and that a deviation either way decreased the number of colonies developing.

The following table from Gage and Phelps shows conclusively the effect of the general composition of the nutrient medium.

TABLE SHOWING PERCENTAGES OF BACTERIA DEVELOPING ON MEDIA OF DIFFERENT COMPOSITIONS.

(Gage and Phelps, 1902.)

Medium.	Days' Count.							
	2	3	4	5	6	7	8	9
Nährstoff agar	19	60	78	85	95	99	99	100
Nährstoff pepton agar	10	22	26	28	30	30	30	30
Pepton agar	11	16	22	23	24	24	24	24
Meat agar	8	13	16	17	17	17	17	17
Plain agar	8	10	13	14	14	14	14	14
Regular agar	7	9	11	11	11	11	11	11
Nährstoff glycerin agar	6	10	11	11	11	11	11	11
Nährstoff meat agar	7	7	8	8	10	10	10	10
Meat gelatin	12	19	24	26	26	26	26	26
Pepton gelatin	7	12	18	20	20	20	20	20
Standard gelatin	8	10	11	12	13	13	13	13
Plain gelatin	1	6	12	13	13	13	13	13
Nährstoff gelatin	5	6	9	11	13	13	13	13

Finally, Whipple (Whipple, 1902) has shown that not only the particular kind of gelatin used but its exact physical condition as affected by sterilization and other previous treatments will materially affect the results obtained. It is evident, therefore, that only the strictest adherence to some standard method can ensure comparable results; the ordinary nutrient gelatin should then in all practical sanitary work be made up in exact accordance with the direction of the A. P. H. A. Committee as given in the Appendix.

Incubation.—"Incubation should take place in a dark, well-ventilated chamber where the temperature is kept substantially constant at 20° and where the atmosphere is practically saturated with moisture."—A. P. H. A. Report. It has been shown by Whipple (Whipple, 1899) and others that the number of bacteria developing in plate cultures is to a certain extent dependent upon the presence of abundant oxygen and moisture. Thus reckoning the number of bacteria developing in a moist chamber at 100 the percentage counts obtained in an ordinary incubator were as follows: 75 when the relative humidity of the incubator was 60 per cent of saturation; 82 when it was 75 per cent; 98 when it was 95 per cent. This source of error may be avoided by the use of ventilated dishes and by the presence of a pan of water in the incubating chamber.

According to American and German practice, plates made for sanitary water analysis are counted at the end of forty-eight hours. French bacteriologists still recommend longer periods, and the following table from Miquel and Cambier (Miquel and Cambier, 1902) shows that many bacteria fail to appear in our ordinary analysis. It is, however, as we have before pointed out, certain peculiar water forms which develop so slowly, sewage bacteria almost without exception being rapid growers. The longer period of incubation is, therefore, not only inconvenient but undesirable, since it obscures the difference between good and bad waters.

EFFECT OF THE LENGTH OF INCUBATION OF WATER BACTERIA IN GELATIN UPON THE NUMBER OF COLONIES DEVELOPING.

(Miquel and Cambier, 1902.)

Length of Incubation.	Colonies Developed.
1 day	20
2 days	136
3 "	254
4 "	387
5 "	530
6 "	637
7 "	725
8 "	780
9 "	821
10 "	859
11 "	892
12 "	921
13 "	951
14 "	976
15 "	1000

Counting.—The number of bacteria is determined by counting the colonies developed upon the plate, either with the naked eye or preferably with a low-power lens. This is actually done by placing the plate upon a glass plate ruled in centimeter squares and over a black tile; or the tile itself may be ruled. The colonies then appear as whitish round or oval specks in and upon the medium. As has already been said, it is desirable that this number should not exceed 200, for when the number is very high the colonies grow only to a small size and make counting laborious and inaccurate, and many do not develop at all. On the other hand, too few bacteria upon the plate probably give inaccurate results also. The greatest accuracy is probably obtained with numbers ranging from 50 to 200.

When it is possible to do so, all the colonies on the plate should be counted. When they exceed 400 or 500 it is often easier and fully as accurate to count a fractional part of the plate and estimate the total number therefrom. This should not be done, however, except in case of necessity.

It is customary in determining numbers to make plates in duplicate, thereby affording a check upon one's own work and enhancing the value of the results through the greater accuracy obtained. Owing to the lack of precision in the method, the limit of experimental error is a wide one. It should be possible, however, for careful manipulators to obtain results within 10 per cent of each other, and a closer agreement than this is hardly to be expected. It has been suggested by the committee of the American Public Health Association to adopt the following mode of expressing results.

NUMBERS OF BACTERIA FROM							
1–50	shall be	recorded	to the	nearest	unit		
51–100	"	"	"	"	"	"	5
101–250	"	"	"	"	"	"	10
251–500	"	"	"	"	"	"	25
501–1,000	"	"	"	"	"	"	50
1,001–10,000	"	"	"	"	"	"	100
10,001–50,000	"	"	"	"	"	"	500
50,001–100,000	"	"	"	"	"	"	1,000
100,001–500,000	"	"	"	"	"	"	10,000
500,001–1,000,000	"	"	"	"	"	"	50,000
1,000,001–5,000,000	"	"	"	"	"	"	100,000

The determination of numbers of bacteria in water in the field has frequently been attempted. Since the labora-

tory method of "plating out" is not applicable for field work the Esmarch tube process has often been employed. This consists in introducing into a tube of melted gelatin or agar 1 c.c. of the water and then rotating the tube until the medium has solidified in a thin layer on the inner wall of the tube. In the writers' opinion, this method is open to several objections and does not give as trustworthy results as the laboratory method, even if the samples have to be several hours in transit between the place of collection and the laboratory.

CHAPTER III.

THE INTERPRETATION OF THE QUANTITATIVE BACTERIOLOGICAL ANALYSIS.

THE information furnished by quantitative bacteriology as to the antecedents of a water is in the nature of circumstantial evidence and requires judicial interpretation. No absolute standards of purity can be established which shall rigidly separate the good from the bad. In this respect the terms "test" and "analysis" so universally used are in a sense inappropriate. Some scientific problems are so simple that they can be definitely settled by a test. The tensile strength of a given steel bar, for example, is a property which can be absolutely determined. In sanitary water analysis, however, the factors involved are so complex and the evidence necessarily so indirect that the process of reasoning much more resembles a doctor's diagnosis than an engineering test.

The older experimenters attempted to establish arbitrary standards, by which the sanitary quality of a water could be fixed automatically by the numbers of germs alone. Thus Miquel (Miquel, 1891) furnished a table according to which water with less than 10 bacteria per c.c. was "excessively pure," with 10 to 100 bacteria, "very

pure," with 100 to 1000 bacteria, "pure," with 1000 to 10,000 bacteria, "mediocre," with 10,000 to 100,000 bacteria, "impure," and with over 100,000 bacteria, "very impure." Few sanitarians would care to dispute the appropriateness of the titles applied to waters of the last two classes; but many bacteriologists have placed the standard of "purity" much lower. The limits set by various German observers range, for example, from 50 to 300. Even Dr. Sternberg (Sternberg, 1892), in a much more conservative fashion, has stated that a water containing less than 100 bacteria is presumably from a deep source and uncontaminated by surface drainage; that one with 500 bacteria is open to suspicion; and that one with over 1000 bacteria is presumably contaminated by sewage or surface drainage. This is probably as satisfactory an arbitrary standard as could be devised, but any such standard must be applied with great caution. The source of the sample is of vital importance in the interpretation of analyses; a bacterial count which would condemn a spring might be quite normal for a river; only figures in excess of those common to unpolluted waters of the same character give the indication of danger. Furthermore, the bacteriological tests are far more delicate than any others at our command, very minute additions of food material causing an immense multiplication of the microscopic flora. This delicacy necessarily requires, both in the process of analysis and the interpretation of results, a high degree of caution. As pointed out in the previous chapter, the touch of a finger or a particle of

dust may wholly destroy the accuracy of an examination. Even the slight disturbance of conditions incident upon the storage of a sample after it has been taken may in a few hours wholly alter the relations of the contained microbic life. It is necessary, then, in the first place to exercise the greatest care in allowing for possible error in the collection and the handling of bacteriological samples; and in the second place, only well-marked differences in numbers should be considered as possibly significant.

In the early days of the science, discussion ran high as to the interpretation of bacteriological analysis; and particularly as to the relation of bacterial numbers to the organic matter present in a water. Different observers obtained inconsistent results, and Bolton (Bolton, 1886) concluded that there was no relation whatever between the chemical composition of a water and its bacterial content. Tiemann and Gärtner (Tiemann and Gärtner, 1889) furnished the key to the difficulty in their statement that there are two great classes of bacteria, the great majority of species normally occurring in the earth or in decomposing organic matter which require abundance of nutriment, and certain peculiar water bacteria which can multiply in the presence of such minute traces of ammonia as are present in ordinary distilled water. Bacteria of the second class under abnormal conditions, as in bottled samples, or, at times, in a well or the basin of a spring, may occur in great numbers where there is but little organic matter. In normal surface-waters, however, such growths have not been recorded as far as we

are aware. Here the numbers of bacteria present will depend, other things being equal, upon the extent to which he water has been contaminated with decomposing organic matter, either by pollution with sewage or by contact with the surface of the ground. The bacterial content will therefore vary as the extent and character of the contamination varies It measures not merely organic matter but organic matter in a state of active decay, and thus, like the ammonias and other features of the sanitary chemical analysis, clearly indicate, fresh organic pollution with the added advantage that the presence of the stable nitrogenous compounds often present in peaty waters introduces no error in the bacteriological analysis.

In judging of a surface-water the student will be aided by reference to the figures given for certain normal sources in Chapter I; the Boston tap water with 50 to 200 bacteria per c.c. (Whipple, 1896) and the water of Lake Zurich with an average of 71 in summer and 184 in winter (Cramer, 1885) may be taken as typical of good potable waters; and numbers much higher than these are open to suspicion, since all contamination whether contributed by sewage or by washings from the surface of the ground is a possible source of danger. The excess of bacteria in surface-waters during the spring and winter months is by no means an exception to the general rule that high numbers are significant, since the peril from supplies of this character is clearly shown by the spring epidemics of typhoid fever which at the times of melting snow visit communities making use of unprotected surface-waters.

Streams receiving direct sewage pollution exhibit a similar excess of bacteria at all times, numbers rising to an extraordinary height near the point of entrance and falling off below as the stream suffers dilution and the sewage organisms perish. Miquel (Miquel, 1886) records 300 bacteria per c.c. in the water of the Seine at Choisy, above Paris, 1200 at Bercy in the vicinity of the city, and 200,000 at St. Denis after the entrance of the drainage of the city. Prausnitz (Prausnitz, 1890) found 531 bacteria per c.c. in the Isar above Munich, 227,369 near the entrance of the principal sewer, 9111 at a place 13 kilometers below the city, and 2378 at Freising, 20 kilometers further down. Jordan (Jordan, 1900), in his study of the fate of the sewage of Chicago, found 1,245,000 bacteria per c.c. in the drainage canal at Bridgeport, 650,000 twenty-nine miles below at Lockport, and numbers steadily decreasing below to 3660 at Averyville, 159 miles below the point of original pollution. Below Averyville the sewage of Peoria enters and the numbers rise to 758,000 at Wesley City, decreasing to 4800 in 123 miles flow to Kampsville.

In ground-waters we have seen that bacteria may be present in considerable numbers, but that they will generally be organisms of a peculiar character, incapable of development on the ordinary nutrient media in the standard time. Thus in forty-eight hours we often obtain counts measured only in units or tens. When higher numbers are present, the general character of the colonies must be taken into account, since besides the slowly-growing forms certain other water bacteria which

require a comparatively small amount of nutriment may multiply at times in a deep well or the basin of a spring. In such a case, however, the appearance of the plates would at once reveal the peculiar conditions, for the colonies would all be of one kind and that distinct from any of the sewage species. Thus Dunham (Dunham, 1889) reports that the mixed water from a series of driven wells gave 2 bacteria per c.c., while another well, situated just like the others, contained 5000, all belonging to a single species common in the air. Plates from polluted water contain, on the other hand, a wide variety of species.

The process of slow sand filtration for the purification of unprotected surface-water is essentially similar to the action which takes place in nature when rain soaks through the ground to appear in wells and springs; and it is in the examination of the effluent from such municipal plants that the quantitative bacteriological analysis finds, perhaps, its most important application. The chemical changes which occur in the passage of water through sand at a rate of 1,000,000 or 2,000,000 gallons per acre per day are so slight as to be negligible. The bacteria present should, however, suffer a reduction of 98 or 99 per cent, and their numbers are, therefore, used as the standard for measuring the efficiency of such filtration plants. At Lawrence, in 1901, Clark found an average of 3017 bacteria per cc. in the raw water of the Merrimac River, while the number present in the filtered water was only 26 (Massachusetts State Board of Health, 1902). Mechanical filtration gives similar results. Fuller at Cincinnati

(Fuller, 1899) records 27,200 organisms per c.c. in the water of the Ohio River between September 21, 1898, and January 25, 1899, while the average content of the effluent from the Jewell filter was 400. In well-managed purification plants the bacteria in the effluent are determined daily, and any deviation from the normal value at once reveals disturbing factors which may impair the efficiency of the process. In Prussia official regulations demand such systematic examinations and prescribe 50 as the maximum number of bacteria allowable in the filtered water. In the same way the condition of an unpurified surface supply may be determined by daily bacteriological analyses and warnings of danger issued to the public, as has been done at Chicago and other cities. In general, any such regular determination of variations from a normal standard furnish ideal conditions for the bacteriological methods; and the detection by Shuttleworth (Shuttleworth, 1895) of a break in a conduit under Lake Ontario by a rise in the bacteria of the Toronto water-supply may be cited as a classic example of its application.

Often, however, the expert is called to pass upon the character of a water of which no series of analyses is available and whose surroundings it may be impossible for him to inspect. It has been said that single bacteriological analyses of this kind are valueless; but this we believe cannot always be maintained. Knowing the normal bacterial range for a given class of waters, even an isolated analysis may show such an excess as to have great significance, as a few practical examples may make clear (Winslow, 1901).

In the spring of 1900 the city of Hartford, Conn., was using a double supply from the Connecticut River and from a series of impounding reservoirs among the hills. A single series of plates showed from 4000 to 7000 bacteria per c.c. in the water of the river, while the reservoir water contained 300 to 900. The abandonment of the river supply followed, and at once the excessive amount of typhoid fever in the city was curtailed.

In the fall of 1900, Newport, R. I., experienced an outbreak of typhoid fever, and when suspicion was thrown upon the public water-supply, chemical analysis of the latter was not wholly reassuring; but there were only 334 bacteria per c.c. in the water from the taps, while a well in the infected district gave 6100. It was no surprise to find, on a further study of the epidemic, that the well was largely at fault and the public supply not at all.

In the case of ground-water the evidence is usually even more distinct. At Framingham, Mass., in 1903, high chlorin content in the public supply, drawn from a filter gallery beside a lake, had led to public anxiety. Five samples from different parts of the system showed averages of 1, 2, 2, 2, and 4 bacteria per c.c.; and taking this in conjunction with the other features of the bacteriological analysis, it was possible to report that any pollution introduced upon the gathering ground had at the time of analysis been entirely removed. In such a case the bacteriological methods give a certainty attainable by no other sanitary process.

CHAPTER IV.

DETERMINATION OF THE NUMBER OF ORGANISMS DEVELOPING AT THE BODY TEMPERATURE.

THE count of colonies upon the gelatin plate measures as we have pointed out, the number of those micro-organisms associated with the decomposition of organic matter wherever it may occur. In this great class, however, there are a few species, like B. subtilis and B. ramosus, which will grow under a great variety of conditions and which are likely to be present with more or less constancy in water, and others which through a semi-parasitic mode of life have become specially adapted to the peculiar conditions characteristic of the animal body. The latter in particular possess the property of developing most actively at the temperature of the human organism, 37° C., which altogether checks the growth of the majority of normal earth and water forms. The determination of the number of organisms growing at the body temperature may throw light, then, on the presence of direct sewage pollution, since the bacteria from the alimentary canal flourish under such conditions, while most of those derived from other sources do not. The count at 37° helps to distinguish contamination by wash of the soil of a virgin woodland from pollution by excreta, since in the latter

case the proportion of blood-temperature organisms is much smaller than in the latter. Furthermore, this method is free from much of the error introduced by the multiplication of bacteria after the collection of a sample, since most of the forms which grow in water during storage cannot endure the higher temperature and consequently do not develop upon incubation.

The body-temperature count must, of course, be made upon agar plates, otherwise the technique is the same which has already been described for the routine quantitative bacteriological analysis. The period of incubation ordinarily adopted by the writers is twenty-four hours, as little development occurs after that time. Difficulty is sometimes caused by the spreading of colonies of certain organisms over the surface of the plate in the water of condensation which gathers; this may be avoided by inverting the plates after the agar is once well set.

Additional evidence as to the character of a water sample may be obtained with little extra labor by adding a sugar and some sterile litmus to the agar medium and observing the fermenting powers of the organisms present, as first suggested by Wurtz (Wurtz, 1892) for the separation of B. coli from B. typhi. It happens that the most abundant intestinal organisms belonging to the groups of the colon bacilli and the streptococci decompose dextrose and lactose with the formation of a large excess of acid, while many other organisms, even if they grow abundantly at the body temperature, are not favored by the presence of the sugar and litmus. The decomposi-

tion of the latter sugar is almost entirely wanting among the commoner saprophytic bacteria, and therefore lactose is most commonly used in making sugar agar, 2 per cent being added to the medium just before the final filtration (between steps 15 and 16 in the standard process of media making given on p. 120). In pouring the plate a cubic centimeter of sterile litmus solution should be added; and in counting, the colonies of the acid-forming organisms will be clearly picked out by the reddening of the adjacent agar. Only those which show this clearly should be considered as significant, since certain bacteria of the hay-bacillus group produce weak acid and faint coloring of the litmus.

When polluted waters are examined in this manner the number of organisms developing on the lactose-agar plate will be very high, almost equalling in some cases the total count obtained on gelatin. Chick (Chick, 1901), using a lactose-agar medium with the addition of one-thousandth part of phenol, found of colon bacilli alone 6100 per c.c. in the Manchester ship canal, 55–190 in the polluted River Severn, and numbers up to 65,000 per gram in roadside mud. In an examination of water from the Charles River above Boston, total 37° counts ranging from 9800 to 16,900 have been found. Two twenty-four-hour examinations of Boston sewage made at the Sanitary Research Laboratory of the Institute of Technology during the summer of 1903 gave an average of 3,660,000 bacteria per c.c. on gelatin at 20° and 2,310,000 per c.c. on lactose agar at 37° with 550,000 acid-formers.

In unpolluted waters not only the absolute number of organisms developing at the body temperature, but its ratio to the gelatin count, is very different. Rideal (Rideal, 1902) states that the proportion between the two counts in the case of a London water in a year's examination was on the average one to twelve. Mathews (Mathews, 1893) in 1893, gave the following figures, the contrast between the ponds and streams which were presumably exposed to pollution on the one hand, and the wells, springs, and taps on the other, being marked.

Sources of Water.	Average Number of Colonies per c.c.	
	Gelatin, 20°.	Wurtz Agar, 37·5°.
Wells, springs	1664	28
Reservoirs	153	43
Ponds	296	95
Taps	242	24
Streams	273	101

In 1903 Nibecker and one of ourselves (Winslow and Nibecker, 1903) made an examination of 259 samples of water from presumably unpolluted sources in Eastern Massachusetts, including public supplies, brooks, springs, ponds, driven wells, and pools in the fields and woods, with a view to testing the value of the body-temperature examination. In many cases the samples showed high gelatin counts, since some of the waters were exposed to surface wash from vacant land, but the average number of organisms developing on lactose agar at 37° was less

RELATION OF 20° AND 37° COUNTS IN SAMPLES OF WATER FROM APPARENTLY UNPOLLUTED SOURCES.

(Winslow and Nibecker, 1903.)

Source of Samples.	Number of Samples.	Gelatin Plates, 20°.	Litmus-lactose-agar Plates, 37°.		Dextrose Broth Tubes.			
		Average Number of Colonies.	Average Number of Colonies.	Plates Showing Red Colonies.	Number of Tubes.	Number of Tubes with Gas.	Number of Tubes with Gas 1–0.	Number of Tubes with Gas 2–1.
Cambridge supply (tap)	5	94	11	0	15	0	0	0
Wakefield and Stoneham supply (tap)	7	59	6	0	21	0	0	0
Lynn supply (tap)	6	16	3	0	18	0	0	0
Brookline supply (tap)	1	.....	18	2	3	0	0	0
Plymouth supply (tap)	6	35	2	0	18	0	0	0
Peabody supply (tap)	3	141	21	0	9	2	2	0
Dedham supply (tap)	6	3,717	1	0	18	0	0	0
Newburyport supply (tap)	6	36	9	0	18	0	0	0
Salem supply (tap)	5	232	14	0	15	0	0	0
Taunton supply (tap)	4	13	2	0	12	0	0	0
Sharon (well) (tap)	3	738	46	2	9	3	0	3
Medford supply (tap)	5	524	8	0	15	0	0	0
Milton supply (tap)	2	4,700	0	0	6	0	0	0
Westerly, R. I., supply (tap)	1	.....	12	0	3	0	0	0
Brooks	61	223	7	0	183	13	13	0
Driven wells	15	18	1	0	45	0	0	0
Springs	32	294	2	0	95	13	13	0
Ponds fed by brooks	15	167	9	0	45	1	1	0
Melted snow	1	.....	4	0	3	0	0	0
Pools in fields	22	365	31	0	66	2	2	0
Pools in woods	22	181	3	0	65	0	0	0
Roadside pools	10	811	4	0	30	2	2	0
Stream, Blue Hill Reservation	1	.....	0	0	3	0	0	0
Flow from rocks	2	47	0	0	6	0	0	0
Ponds fed by springs	6	188	2	0	18	0	0	0
Drainage from manured pasture	1	1,235	27	0	3	0	0	0
Swamps	3	269	6	0	9	5	5	0
Rain-water after twelve hours' heavy fall	7	.....	2	0	21	0	0	0
Shallow well in Lynn woods	1	15	1	0	3	0	0	0
Totals	259			4	775	41	38	3

than 8 per c.c. The highest individual counts obtained, as will be seen by reference to the table on the preceding page, were 95 in a meadow pool, 83 in a brook, and 74 in a barnyard well, the latter probably actually polluted. Only two samples in the whole series, one from the well above mentioned, gave any red colonies on the agar plates.

Thus it is clear that organisms growing at the body temperature and those fermenting lactose are not numerous in normal waters, the total count rarely exceeding 50, with acid producers generally entirely absent. On the other hand, the numbers on the litmus-lactose-agar plate will be likely to run into hundreds with a good proportion of red colonies when polluted waters are examined. The method is, therefore, one of the most useful at the disposal of the bacteriologist. It yields results within twenty-four hours, and the conclusions to be drawn from it are definite and clear.

CHAPTER V.

THE ISOLATION OF SPECIFIC PATHOGENES FROM WATER.

THE discovery of the organisms which specifically cause the infectious diseases naturally led to the hope that their isolation from polluted water might become the most convincing proof of its sanitary quality. The typhoid bacillus and the spirillum of Asiatic cholera were in this connection of paramount importance, and to the search for them many investigators devoted themselves.

In the earlier examinations of water for the typhoid bacillus an attempt was made to use media which especially favored the growth of the microbe sought for, or to begin with some process of "enrichment" in which the sample was incubated under conditions which would favor the growth of the pathogenic organisms while checking the development of the common water bacteria. It was apparent that the body temperature and the presence of a slight excess of free acid furnished such conditions, and most of the methods suggested rest upon these principles. The most general perhaps is that of Parietti (Parietti, 1890), which consists in the addition of the water to a series of broth tubes containing increas-

ing amounts of a solution of 4 per cent hydrochloric acid and 5 per cent phenol. From tubes in which growth occurs after twenty-four hours at 37°, the organisms present may be isolated in pure cultures by some plating method and identified by subcultures.

The great difficulty with the enrichment processes is that the conditions which favor the multiplication of the typhoid bacillus are suited in an even higher degree to B. coli and other intestinal organisms. Being present in almost all cases in much higher numbers than B. typhi, these germs develop most abundantly, and effectually mask any disease germs originally present. In order to obviate this difficulty, Hankin (Hankin, 1899), after adding successively increasing portions of Parietti solution to tubes inoculated with the water to be tested, selected the second highest tube of the series in which growth occurs for the inoculation of a new set, finally plating as above. He believed that the chance for overgrowth in this method is somewhat decreased; but in the hands of other investigators it has not met with marked success. Klein (Thomson, 1894), in his investigations, made use of the Berkefeld filter to concentrate the organisms in the sample. Some recent observers have abandoned the enrichment process altogether and recommend direct plating upon phenolated gelatin or on the Elsner (Elsner, 1896) medium made by adding 10 per cent of gelatin and 1 per cent of potassium iodide to an infusion of potato whose reaction has been adjusted to 30 on Fuller's scale. In all cases, however, the chance

of success is small; as is well shown by the experiments of Laws and Andrewes (Laws and Andrewes, 1894), who entirely failed to isolate the typhoid bacillus from the sewage of London and found only two colonies of the organism on a long series of plates made from the sewage of a hospital containing forty typhoid patients. So Wathelet (Wathelet, 1895) found that of 600 colonies isolated from typhoid stools and having the appearance characteristic of B. coli and B. typhi only 10 belonged to the latter species.

At the other end of the process the identification of the pure cultures isolated is again subject to considerable uncertainty. The typhoid bacillus belongs to a large group which contains numerous varieties differing from each other by minute degrees. The inability to reproduce the disease by inoculation in available test animals owing to their natural immunity is a serious drawback; and the specific biochemical characters of the organism are, as it happens, mostly negative ones, as shown by comparison with B. coli, to which it is supposed to be allied.

COMPARISON OF THE CHARACTERS OF B. COLI AND B. TYPHI.

(Horrocks, 1901.)

C. coli.	*B. typhi.*
(1) Surface Colonies, Gelatin Plates.—Thicker, and grow more rapidly than those of B. typhi. After forty-eight hours' incubation at 22° C. they are usually large and characteristic.	(1) Much thinner than those of B. coli, and grow more slowly. After forty-eight hours' incubation at 22° C. they are hardly visible to the naked eye.

C. coli.	*B. typhi.*
(2) Gelatin-stab.—Quick growth on the surface and along the line of inoculation.	(2) Slow growth on the surface like the colonies; along the line of inoculation the growth is much thinner, and often ends below in a few white points consisting of discrete colonies.
(3) Gelatin-slope.—Thick broad grayish-white growth with a crenated margin.	(3) Thin narrow grayish-white growth, crenated margin not marked as in B. coli.
(4) Witte's Peptone and Salt Solution.—Indol produced.	(4) No formation of indol.
(5) Milk.—Coagulated.	(5) Unchanged after a month.
(6) Litmus-whey, one week at 37° C. Acid produced, usually requiring from 20 to 40 per cent of $\frac{N}{10}$ alkali to neutralize it.	(6) Very small amount of acid produced, requiring not more than 6 per cent of $\frac{N}{10}$ alkali to neutralize it.
(7) Neutral-red Glucose-agar.—Marked green fluorescence.	(7) No change.
(8) Glucose-gelatin and Lactose-gelatin Shake Cultures, and Glucose-agar-stab.—Marked gas formation.	(8) No gas formation.
(9) Gelatin, 25 per cent, incubated at 37° C.—Thick film appears on the surface.	(9) No film appears on the surface, but a general growth takes place throughout the tube.
(10) Potato.—As a rule, a thick yellowish-brown growth.	(10) Thin transparent growth hardly visible to the naked eye.
(11) Proskauer and Capaldi's Media. No. I, after twenty hours growth, medium acid. No. II, Growth, medium neutral or faintly alkaline.	(11) No. I, no growth or change in the reaction of the medium. No. II, Growth, medium acid.
(12) Nitrate-broth.—Nitrate reduced to nitrite.	(12) Reduction of nitrate not so marked.
(13) Microscopical Appearances.—A small bacillus often like a coccus, not motile as a rule.	(13) Usually longer than B. coli; highly motile, with a quick serpent-like movement.
(14) Flagella.—Usually 1 to 3, short and brittle; sometimes 8 to 12, long and wavy.	(14) Usually 8 to 12, long and wavy.

(15) Agglutination.—As a rule, no agglutination with a dilute anti-typhoid serum.	(15) Marked agglutination with dilute anti-typhoid serum.

Of the many observers who have reported the isolation of the typhoid bacillus from water all but the most recent are quite discredited, on account of the insufficiency of their confirmatory tests, and even the latest results should be received with caution. Since the introduction of the Widal (Widal, 1896) reaction, founded on the fact that typhoid bacilli examined under the microscope in the diluted blood-serum of a typhoid patient lose their motility and "agglutinate" or clump together, an important aid has been furnished in the diagnosis. Yet serum tests are notably erratic, and insufficient to identify an organism without an exhaustive study of biochemical reactions, especially as many organisms are agglutinated by typhoid serum in a more or less dilute solution. The discovery of the Bacillus dysenteriæ of Shiga,* which closely resembles the typhoid bacillus, has made the identification of the latter more dubious than ever.

It seems probable that in some recent cases the typhoid bacillus has indeed actually been isolated from polluted water, as by Kübler and Neufeld (Kübler and Neufeld, 1899), who examined a farmhouse well at Neumark in 1899, and Fischer and Flatau (Fischer and Flatau, 1901), who discovered an organism responding to a most exhaustive series of tests for the typhoid bacillus in a well at

* For an account of the Biology of B. dysenteriæ the student is referred to an article by Dombrowsky, 1903.

Rellingen in 1901. In these cases the water was directly plated upon Elsner's medium or phenolated gelatin with no preliminary process of enrichment.

The search for the typhoid bacillus is usually suggested when an outbreak of the disease has cast strong suspicion upon some definite source of water-supply. By the time an epidemic manifests itself, however, the period of the original infection is long past, and the chances are good that any of the specific bacilli once present will have disappeared. While elaborate experiments have shown that B. typhi may persist in sterilized water for upwards of two months and in unsterilized water from three days to several weeks, the number of the organisms present is always very rapidly reduced (Frankland, 1894). Epidemiological evidence confirms the results of Laws and Andrewes which teach that the number of typhoid bacilli even in polluted water probably is never very great, while the fate of Lowell and Lawrence in 1890–91 seems strongly to demonstrate that even a small number of virulent organisms can bring about an almost wholesale infection. Indeed if the virulent organism were as abundant as some recent results would indicate (Remlinger and Schneider, 1897), the human race would long since have been exterminated. On the whole it seems that since a positive result is always open to serious doubt, and a negative result signifies nothing, the search for the typhoid bacillus itself, however desirable theoretically, cannot be regarded at present as generally profitable.

The isolation of the cholera bacillus from water can

probably be accomplished with somewhat less difficulty than is encountered in the case of B. typhi. Schottelius (Schottelius, 1885) was the first to point out the necessity for growing this organism in an alkaline medium, and Loeffler (Loeffler, 1893) found that its isolation from water could be successfully accomplished by adding 10 c.c. of alkaline peptone broth to 200 c.c. of the infected water and incubating for twenty-four hours at 37°, when the organism could be found at the surface of the medium.

Somewhat earlier than this Dunham (Dunham, 1887) had made a special study of the chemical reactions of the cholera bacillus and found that the organism would grow abundantly in a solution containing 1 per cent peptone and .5 per cent salt (Dunham's solution), producing the "cholera-red or nitroso-indol reaction." This medium was brought into practical use by Dunbar (Dunbar, 1892), who succeeded in isolating the organisms from the water of the Elbe in 1892, during the cholera epidemic at Hamburg.

Koch (Koch, 1893) prescribed the following method for the isolation of the organism from water:

To 100 c.c. of the water to be examined is added 1 per cent peptone and 1 per cent salt. The mixture is then incubated at 37°. After intervals of ten, fifteen, and twenty hours the solution is examined microscopically for comma-shaped organisms, and agar plate cultures are made which are likewise incubated at 37°. If any colonies showing the characteristic appearance of the cholera bacillus are found, these are examined micro-

scopically, and if comma-shaped organisms are present, inoculations are made into fresh tubes to be further tested by means of the indol reaction and by inoculation into animals. The existence of other spirilla of some pathogenic power renders necessary the greatest care and caution in claiming positive isolations. That no great improvement on Koch's method has been made during the last ten years seems apparent from the statements of Kolle and Gotschlich (Kolle and Gotschlich, 1903), who employed "the peptone method with subsequent agar cultivation" in the isolation of the organisms from fæces of cholera patients during the epidemic in Egypt in 1902, and who have made notable epidemiological and clinical researches upon this disease.

Other pathogenic organisms have been isolated from waters, according to the accounts of numerous investigators, but from the sanitary point of view the typhoid and cholera bacilli are of most importance since these are manifestly the germs of disease most likely to be disseminated through this medium. For the detection of B. anthracis and other spore-forming pathogenic bacteria which may at times gain access to water from stockyards, slaughter-houses, etc., the method suggested by Frankland (Frankland, 1894) may be adopted. The water to be examined is heated to 90° for two minutes and then plated, the characteristic colonies of the anthrax organism being much more easily discerned after the destruction of the numerous non-sporing water bacteria. Again, water is sometimes the means of distributing the germs of

dysentery and diarrhœa, as shown by the decrease of these diseases in Burlington, Vt. (Sedgwick, 1902), and other communities where pure water-supplies have been substituted for polluted ones. It is possible that the examination of water for the B. dysenteriæ may in the future help to throw important light on the sanitary condition of a water.

CHAPTER VI.

METHODS FOR THE ISOLATION OF THE COLON BACILLUS.

THE Bacillus coli was first isolated by Escherich (Escherich, 1884) from the fæces of a cholera patient. It was subsequently found to be a normal inhabitant of the intestinal tract of man and many other animals and to occur regularly in their excretions, and on this account it became of the highest interest and importance to sanitarians, since its presence in water-supplies was regarded as direct evidence of sewage pollution.

This organism may be described as a short, usually motile rod, with diameter generally less than one micron and exhibiting no spore formation. It forms thin irregular translucent films upon the surface of gelatin, called "grape-leaf colonies" by the Germans, produces no liquefaction, and gives a wire-nail-like growth in stick cultures. It forms a white translucent layer of characteristic appearance upon agar, produces a more or less abundant, moist, yellowish growth on potato, and turbidity and some sediment in broth; it ferments dextrose and lactose with the formation of gas of which the ratio is $\frac{H}{CO_2}=\frac{2}{1}$, according to most investigators; one variety

ferments saccharose with a gas ratio $\frac{H}{CO_2}$ approaching $\frac{3}{2}$, and another does not. As a rule, a strong acid reaction is developed in all sugar-containing media. The organism reduces nitrates to nitrites and sometimes to ammonia. It coagulates casein in litmus milk, and reduces the litmus with subsequent slow return of the color (red), and forms indol in peptone solution. Many cultures of this organism are fatal to guinea-pigs when the latter are inoculated subcutaneously with ½ c.c. of a twenty-four-hour bouillon culture, and most cultures produce death when this amount is inoculated intraperitoneally. Although not a spore-forming bacillus, and in general not possessing great resistance against antiseptic substances, B. coli seems to be less susceptible to phenol than are many other forms, especially certain water-bacteria.

The Wurtz litmus-lactose-agar plate (Wurtz, 1892), as noted in Chapter IV, furnishes one ready method for the isolation of B. coli from water, and it was used by Sedgwick and Mathews for the purpose as early as 1893 (Mathews, 1893). The process is based upon the fact already alluded to, that B. coli readily ferments lactose with the formation of acid. If, therefore, plates are made with agar containing both lactose and litmus, the colon colonies develop as red spots in a blue field. Since organisms other than B. coli may also develop red colonies, it becomes necessary further to examine the red colonies in order to prove that they are made up of colon bacilli.

This is done by fishing from isolated suspicious-looking colonies, replating and inoculating into the usual media for diagnostic work.

For success in examining polluted waters by this method it is necessary to get a sufficient dilution so that colonies may be well isolated, and to this end it is advisable that a number of different dilutions be employed, a series of plates being prepared from each. Under any conditions the detection of the colon bacillus is seriously hampered by the development of other forms. Certain observers have therefore added phenol to the agar medium, combining the effect of high temperature and an antiseptic to check the growth of water-bacteria. Copeland for this purpose added to his tubes .2 c.c. of a 2% solution of phenol (Copeland, 1901). Chick (Chick, 1900) found that 1.33 parts of phenol in 1000 materially decreased the number of colon bacilli which would develop, while 1 part gave very satisfactory results, the plates showing pure cultures of B. coli.

The test for the colon bacillus may, however, be made still more delicate by a preliminary enrichment of the sample by growth in a liquid medium for twenty-four hours at 37°, thus greatly increasing the proportion of these organisms present before plating. As suggested in the classic researches of Theobald Smith (Smith, 1892), this method may be made approximately quantitative by the inoculation of a series of tubes with measured portions of the water. If, for example, of ten tubes inoculated each with $\frac{1}{100}$ of a cubic centimeter, four show

the B. coli, we may assume that some 40 of these organisms were present to the cubic centimeter. Irons (Irons, 1901), in a comparative study of various methods for the isolation of B. coli, showed that the preliminary enrichment frequently gave positive results when the results of the direct use of the agar plate were negative and concluded that "where the waters to be examined are much polluted, or where the amount of B. coli is small and the colony count large, the lactose plate for plating water direct is inferior to the dextrose fermentation-tube." Gage obtained similar results (Gage, 1902). The method of direct plating in phenol-agar is quicker and there is a certain danger that colon bacilli originally present may be overgrown and killed out in the enrichment process. If the incubation is not too prolonged, however, this need not occur.

The medium ordinarily used for the preliminary enrichment is ordinary broth to which 2.5 per cent of dextrose has been added, and the reaction brought to the neutral point. Into each of a number of fermentation-tubes of this medium a measured quantity of the water to be examined is inoculated, and the culture is incubated for twenty-four hours at 37.5° C. At the end of this time the tubes are examined for gas formation. If gas is found, a small amount of the culture should be added, after suitable dilution, to litmus lactose agar and plated.

With polluted waters it will be found advantageous to plate out on the first appearance of gas (4–8 hours). It has been shown by one of us (Prescott, 1902[b]) that a very

rapid development of B. coli takes place in the first few hours after dextrose solutions are inoculated with intestinal material, and a nearly pure growth of colon bacilli often results, while other bacteria multiply more slowly. With highly polluted waters gas formation will probably begin within twelve hours, but with fewer colon bacilli present the duration must be increased. If the period of incubation be too long continued, trouble in the subsequent steps of the isolation may be encountered because of the overgrowth of B. coli by the sewage streptococci, which are almost invariably present, and which by their greater acid-producing powers may check the growth of the colon bacilli.

As has already been stated, phenol has less inhibitory action upon B. coli than upon normal water-bacteria, hence a broth containing this substance may be employed for preliminary enrichment; and this medium has been used in place of dextrose broth for many of the studies made in connection with the Chicago drainage canal (Reynolds, 1902). Phenol broth consists of ordinary broth to which 0.1 per cent phenol is added, and the method of procedure is to add 1 c.c. of the water to 10 c.c. of the sterilized phenol broth and incubate at body temperature for twenty-four hours. Litmus-lactose-agar plates are then made and the examination of the red colonies carried on as described for the dextrose-broth method. The dextrose broth furnishes, however, a much more delicate test than the carbol broth when the num-

ber of colon bacilli present is small, as is clearly shown by the following table from Irons.

PROPORTION OF POSITIVE RESULTS IN TESTS OF POLLUTED AND UNPOLLUTED WATERS BY DEXTROSE FERMENTATION-TUBE AND CARBOL-BROTH METHODS.

(Irons, 1901.)

	Dextrose Fermentation-tube.			Carbol-broth Method.		
	+	−	?	+	−	?
Polluted waters	33	31	5	38	30	1
Relatively unpolluted waters	56	38	25	37	61	21

Furthermore, the dextrose fermentation-tube possesses a second advantage in the fact that a decisive negative test may be obtained within twenty-four hours of the original planting of the sample. If no gas is formed in the tube, we commonly assume that B. coli is absent and carry the examination no farther.

When it is desired to examine samples larger than 1 c.c. for B. coli it becomes necessary to modify the enrichment process by adding the nutrient material to the water instead of the reverse. For this purpose phenol dextrose broth (consisting of broth with 10 per cent dextrose, 5 per cent peptone, and .25 per cent phenol) may be added to the sample of water to be enriched as suggested by Gage (Gage, 1901). Generally 10 c.c. of the broth is added to 100 c.c. of the water. The sample is then incubated at 37° for twenty-four hours, and if at the end of that time growth has taken place, a cubic centi-

meter is inoculated into a dextrose tube. If this tube shows gas formation after twenty-four hours at 37°, a litmus-lactose-agar plate is made and the other diagnostic tests applied.

Our experience has shown that it is not specially advantageous to apply the colon test in such large samples, since the significance of B. coli when present in numbers less than 1 per c.c. is extremely doubtful. On the other hand the danger of overgrowth is greatly increased by this process and negative results may often be obtained when the organisms are really present. Hunnewell and one of ourselves (Winslow and Hunnewell, 1902[b]) found that of 48 samples of certain polluted river waters 18 gave B. coli when 1 c.c. was inoculated directly into dextrose broth, while in only 4 cases was a positive result obtained after preliminary treatment of 100 c.c. in carbol broth. In 153 samples from presumably unpolluted water B. coli was found 5 times in 1 c.c. and 11 times by the examination of the larger sample. The authors, therefore, concluded as follows:

"It appears evident that the use of large samples in applying the colon test to the sanitary analysis of drinking-water is not advantageous. In comparing the results of the tests in 1 c.c. and in 100 c.c., it will be noted that the proportion of lactose fermenting organisms and of colon bacilli in the unpolluted waters was more than doubled in the latter; thus waters of good quality are more likely to be condemned by the use of large samples. On the other hand, in the polluted waters a considerable propor-

tion of the colon bacilli originally present were lost during the incubation of the large samples, so that waters of bad quality actually appeared to better advantage by the use of 100 c.c. with preliminary incubation in phenol broth."

Similarly Whipple (Whipple, 1903) notes that 2.9 per cent of some samples of water examined by him gave positive tests with .1 c.c. but not with 1 c.c., while 4.3 per cent gave positive tests with .1 c.c. or 1 c.c. and negative tests with 10 c.c. Again, in another series of samples examined, of those which gave positive samples in smaller portions 5.3 per cent were negative in 10 c,c., 4.7 per cent in 100 c.c., and 7.7 per cent in 500 c.c.

In all practical processes of examining water for B. coli one essential step is the isolation of pure cultures upon the litmus-lactose-agar plate, whether the plate be inoculated from the water direct or from a preliminary enrichment culture. In the first case a measured quantity of water must be added. In the second case, since the enrichment-tube was inoculated with a known amount of water all further work is purely qualitative and it is only necessary to obtain such a number of colonies upon the lactose plate that the isolation of a pure culture shall be easy. In practice the following procedure has been found generally successful. After the dextrose-tubes have been incubated for twelve to twenty-four hours at 37°, from those which show gas, one loopful is carried over to a tube containing 10 c.c. of sterile water, and of this water one loopful is taken for the inoculation of the plate. Ordinarily this will give colonies which are sufficiently

well separated, but a second plate, inoculated from the dilution water with a straight needle instead of a loop, furnishes a desirable safeguard.

The litmus-lactose-agar plates made in this manner should be incubated for from twelve to twenty-four hours at the body temperature (37°), at the end of which time if B. coli is present red colonies upon a blue field will be visible. If a pure culture of B. coli is obtained, the litmus-lactose-agar plate may become blue again after forty-eight hours owing to the formation of amines and ammonia by the action of the bacteria upon the nitrogenous matter present. If the dilution is too low, the resulting colonies will be small and imperfectly developed, making it difficult to be sure of pure cultures for the subsequent tests. A great number of colonies will also prevent the change of reaction from acid back to alkaline. Since many bacteria ferment lactose with the formation of acid, it is erroneous to regard all colonies as those of B. coli; in all cases several colonies from each plate should be isolated upon agar streaks and further studied in subculture.

In the selection of those red colonies which are to be fished from the litmus-lactose-agar plate the appearance of the growths must be closely noted. A colony of irregular contour, surrounded by a very faint area of reddening, will probably belong to some member of the B. mycoides group (Winslow and Nibecker, 1903); small, compact, bright-red colonies are characteristic of the streptococci, and Gage and Phelps (Gage and Phelps, 1903) have pointed out that of these there are two types, one of a

brick-red color, and of such consistency as to be readily picked up by the needle-point and the other smaller and of an intense vermilion color. The colonies of the colon bacillus are usually well formed, pulvinate on the surface and fusiform when growing deeper down.

The agar streak made from the litmus-lactose-agar plate shows after twenty-four hours certain marked characteristics. The most distinct types are two, the abundant, first translucent, later whitish and cheesy growth, covering nearly the whole surface of the agar, characteristic of B. coli and its allies, and a very faint growth, either confined strictly to the streak or made up of faint isolated colonies, dotted here and there over the surface. The latter cultures are typical of the sewage streptococci, and a microscopic examination will generally settle their status at once. Of the more luxuriant growths, some of which are stringy to the needle many will generally prove to be atypical, and if any of the weakly fermenting forms (B. mycoides) are present, a dull wrinkled growth will be produced.

Having submitted the sample of suspected water to a preliminary enrichment process, and having isolated pure cultures of suspicious organisms from the litmus-lactose-agar plate, the third step is the examination of the specific reactions of the organisms thus obtained. Just what characters to use in defining the "colon bacillus" is a matter of prime importance. The whole question of species among the bacteria is an extremely complex one, since around each definite species are grouped forms

differing from the type in one or two of its characteristics.

As Whipple says (Whipple, 1903), "The type form of Bacillus coli is one which can be defined within reasonably narrow limits, but when the organism has been away from its natural habitat for varying periods of time, and has existed under abnormal conditions, its ability to react normally to the usual tests appears to be greatly impaired. Its power to reduce nitrates may be lost, or on the other hand may be increased; its power to produce indol may be lost, or on the other hand, it may be increased; its power to coagulate milk, even, is sometimes reduced, although seldom entirely lost; its power to ferment carbohydrates may be altered so that the amount of gas obtained in a fermentation-tube, as well as its ratio of H to CO_2, is quite abnormal. But in spite of all these facts, the bacillus tested may have been originally a true Bacillus coli."

The more of such atypical forms which are included the greater will be the number of positive isolations. At present our definitions must be more or less arbitrary; each observer will consider as true colon bacilli those which fulfil his particular set of tests, and will class as pseudo-colon organisms those which do not. If we find, having established such an arbitrary standard, that the colon bacillus, as determined by it, is found in waters known to be polluted, and not, as a rule, in those known to be free from pollution, the sanitarian can afford to ignore the theoretical question of specific values and

make confident use of the practical test. It is, of course, highly desirable that some standard set of reactions should be commonly adopted by sanitary bacteriologists; and it is to be hoped that some such uniform scheme may soon be drawn up by the Society of American Bacteriologists or by the American Public Health Association. At present the plan developed by the Massachusetts State Board of Health (Massachusetts State Board of Health, 1899) is most widely prevalent in this country. It involves the use of six simple, definite, positive tests—the growth in gelatin, lactose-agar, dextrose broth, milk, nitrate solution, and peptone solution.

For recording the results of the various tests applied, the appended blank form has been in use at the Massachusetts Institute of Technology. On the upper part of the sheet are noted the results of the gelatin count and the litmus-agar count at 37°. In the second case the number of acid-formers is placed in brackets after the total numbers. The lower part is used for the B. coli isolation.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY.

BACTERIOLOGICAL EXAMINATION OF WATER.

Sample No.	Date of collection
Examined by	Hour of collection
Place of collection	Remarks

QUANTITATIVE ANALYSIS.

Gelatin-plate cultures. 48 hours	No. colonies per c.c.	Agar-plate cultures at 37°. No. colonies per c.c. 24 hours
Average		Average
Remarks		Remarks

QUALITATIVE ANALYSIS.

Preliminary fermentation-tube
Litmus-lactose-agar plates
Agar streak culture
Fermentation-tube
Nitrate test
Milk test
Indol reaction
Gelatin stab culture
Remarks

Opposite the words "Preliminary fermentation-tube" the results of the first enrichment test in the five or ten tubes inoculated are recorded by plus or minus signs. Under the columns headed by plus signs the presence or absence of typical red colonies on the litmus-lactose-agar plate is similarly indicated. The third line serves for the results of the microscopic and macroscopic appearance of the agar streak. If this gives the proper type of growth and rod-shaped organisms are found, a dextrose fermentation-tube, a nitrate tube, a milk tube, a peptone tube, and a gelatin stab are inoculated from it.

After twenty-four hours incubation at 37°, the first three of these subcultures are examined and the results recorded in the proper columns by plus and minus signs. The amount of gas in the closed arm of the dextrose tube may be conveniently measured by the Frost gasometer (Frost, 1901). Then a few centimeters of strong sodium or potassium hydrate are added and mixed with the broth by cautiously tipping the tube and a second measurement determines the amount of gas absorbed (assumed to be CO_2). The gas should first fill from a third to two-

thirds of the closed arm and about one-third of this should be absorbed. The nitrate tube is tested for nitrites by adding a drop of the following solutions in succession:

A.	Sulphanilic acid	.5 gram
	Acetic acid (25% sol.)	150.0 c.c.
B.	Naphthylamine chloride	.1 gram
	Distilled water	20.0 c.c.
	Acetic acid (25% sol.)	150.0 c.c.

A red or violet coloration indicates the presence of nitrites. The milk tube is merely heated to boiling over the free flame; if coagulation occurs the test is considered positive.

After seventy-two hours incubation at 37° the peptone solution is examined for indol by adding 1 c.c. of a .02 per cent solution of sodium or potassium nitrite and 1 c.c. of a 1 to 1 solution of sulphuric acid. Both the tube and the reagents should be cooled on ice before mixing, and the tube should be left in a cool place for an hour afterward to allow time for the characteristic rose-red color of nitroso-indol to develop. The gelatin tube is kept at 20° for seven days according to the procedure adopted at the Massachusetts Institute of Technology. It seems undesirable in practice to prolong the test much beyond this point, although some slowly liquefying organisms are doubtless included, which would be thrown out by a longer incubation. The extent of this source of error as well as the relative importance of the various other diagnostic tests is well shown in the following table of

PERCENTAGE OF CULTURES PASSING VARIOUS TESTS IN THE ROUTINE EXAMINATION FOR B. COLI AT THE LAWRENCE EXPERIMENT STATION OF THE MASSACHUSETTS STATE BOARD OF HEALTH (GAGE AND PHELPS, 1903).

Source of Culture.	Per Cent of Preliminary Fermentations Passing Wurtz Agar Plate.	Agar Streak.					Number of Cultures Inoculated into Confirmatory Media.	Per Cent of Cultures.															Per Cent of Total Cultures Called B. Coli.
		Number of Cultures Made.	Per Cent of Cultures.					Fermentation-tube, Dextrose Broth.		Coagulate Milk.	Reduce Nitrates.	Produce Indol.	Gelatin Stab.										
													Not Liquefied.		Liquefied.								
															Time.				Form.				
			Characteristic.	No Growth.*	Dull-wrinkled.	Viscous.		Produce Gas.	Produce Scum.				Characteristic.	Wrinkled.	4 Days.	7 Days.	10 Days.	14 Days.	Funnel.	Cup.	Stratiform.	
B. coli	..	....	+	0	0	0	....	+	0	+	+	+	+	0	0	0	0	0	0	0	0		
Merrimac River	..	273	86	0	13	1	236	81	13	86	82	73	69	1	21	25	28	30	10	4	16	59
Filtered water	76	1485	83	9	4	4	1236	84	3	90	76	71	83	4	4	7	9	13	5	5	3	58
Sewage, etc	..	160	87	4	8	1	140	75	0	88	70	75	84	1	9	9	10	15	6	2	7	59
Shellfish	72	312	64	33	2	1	210	68	3	91	67	75	84	0	9	9	9	16	6	2	8	43
Spring-waters	78	53	74	0	4	23	39	56	0	67	67	59	72	0	15	18	28	28	20	0	8	41
Ice	73	79	72	16	8	0	557	67	5	88	42	88	63	7	25	25	26	30	5	4	21	48
Totals	75	2362	81	11	5	3	1908	80	4	88	74	71	80	3	8	10	13	17	6	5	6	58

* Including cultures which failed to grow on agar and streptococcus cultures, giving a very scanty, non-characteristic growth.

the results obtained at Lawrence during a period of eighteen months.

It should be noted that considerable differences often appear in these biochemical reactions between tubes of the same batch of a medium, inoculated with approximately the same amount of the same culture. This has been shown very markedly for the amount of the gas and the proportion of carbon dioxide in the dextrose tube by Fuller and Johnson (Fuller and Johnson, 1899), Pennington and Küsel (Pennington and Küsel, 1900), Gage (Gage, 1902), and one of ourselves (Winslow, 1903). Variations in the nitrate reduction are often even more marked, one tube, perhaps, showing a strong reaction and another none. In important cases, therefore, it is desirable to inoculate the subcultures in duplicate.

CHAPTER VII.

SIGNIFICANCE OF THE PRESENCE OF B. COLI IN WATER.

TEN years ago the B. coli of Escherich occupied a position of very great prominence in the eyes of sanitarians. If it was not considered to be in itself a dangerously pathogenic germ, it was at least regarded as a suspiciously close relation of the typhoid organism. At this time, therefore, the alleged presence of either of these forms was quite sufficient to condemn a water-supply.

Investigation soon showed, however, that the Bacillus coli was by no means confined to the human intestine. Dyar and Keith (Dyar and Keith, 1893) found it to be the prevailing intestinal form in the cat, dog, hog, and cow. In the goat and rabbit they reported that no single organism was constantly present; and in the case of the horse, the place of the colon bacillus was taken by a new form, described by the authors under the name of B. equi intestinalis. About the same time, Fremlin (Fremlin, 1893) found colon bacilli in the feces of dogs, mice, and rabbits, but not in those of rats, guinea-pigs, and pigeons. Smith (Smith, 1895) recorded the presence of the same organism, in almost pure cultures, in the intestines of dogs,

cats, swine, and cattle; and he also found it in the organs of fowls and turkeys after death. Brotzu (Brotzu, 1895) reported B. coli and allied forms as very abundant in the intestine of the dog; and Belitzer (Belitzer, 1899) still more recently isolated typical colon bacilli from the intestinal contents of horses, cattle, swine, and goats. Russell and Bassett (Russell and Bassett, 1899) stated that studies made by E. B. Hoag in Professor Russell's laboratory indicated the presence of bacilli of the colon group, fermenting dextrose with a gas formula of $\frac{2}{1}$, in the feces of "a considerable number of different species of mammalia, as well as that of birds, fish, etc.," while similar organisms with the inverted gas formula $\frac{1}{2}$ were considered by the same authors to be characteristic of decomposing organic matter of vegetable origin, free from suspicion of fecal contamination. Moore and Wright (Moore and Wright, 1900) recorded the finding of the colon bacillus in the horse, dog, cow, sheep, and hen; and in a later report (Moore and Wright, 1902) they noted its occurrence in swine and in some, but not all, the specimens of rabbits examined. In frogs it was not found. Amyot (Amyot, 1902) failed to find B. coli in the intestines of 23 fish representing 14 species. The possibility that colon bacilli may be introduced into unpolluted waters through the agency of fish was considered again by Johnson, in a paper read at the Washington meeting of the American Public Health Association and not yet published. Colon bacilli were found present in the intestinal tract of fish taken from polluted waters, and

the conclusion was drawn that the migration of fish from a contaminated stream or lake to an unpolluted one may explain the occasional finding of B. coli in small samples, or the more regular detection of it in large volumes of the water.

Many bacteriologists have gone much further and affirmed that the colon bacillus was not a form characteristic of the intestine at all, but a saprophyte having a wide distribution in nature. The first of this school, perhaps, was Kruse (Kruse, 1894), who in 1894 protested against the arbitrary conclusions drawn from the colon test as then applied. He pointed out that the characters usually observed marked, not a single species, but a large group of organisms. As ordinarily defined, he added, "the Bacterium coli is in no way characteristic of the feces of men or animals. Such bacteria occur everywhere, in air, in earth, and in the water, from the most different sources." Even if the relations to milk and sugar media be considered, "micro-organisms with these characteristics are also widespread." Dr. Kruse gave no experimental data on which his opinion was based. In the same year Beckmann (Beckmann, 1894) isolated a bacillus which he identified by pretty thorough tests as B. coli from the city water of Strassburg, a ground-water which he believed could by no possibility be subject to fecal contamination. Large quantities of water were used for the isolation.

Refik (Refik, 1896) recorded the constant presence of colon bacilli in water of all sorts, public supplies, wells, cisterns, and springs in the neighborhood of Constanti-

nople, but the only characters which these "colon bacilli" exhibited in common were the "classical growth" upon potato, the possession of less than 8 cilia, and the power of active development on certain media upon which the typhoid bacillus did not grow. A more careful and significant piece of work on the same line was published by Poujol in the succeeding year. This author reported (Poujol, 1897) the isolation of B. coli from 22 out of 34 waters studied by him in relation to their use as public supplies. The waters were from various sources—springs, wells, and rivers—but all were of fair quality and many quite free from any possibility of contamination. Samples of 100 c.c. were used for analysis; in the only case in which a smaller amount was also tested, broth inoculated with 10 drops of the water and placed at 45° C. remained sterile. The author concluded that "fecal contamination can only exceptionally be invoked to explain the presence of B. coli in water. As the bacteria of the subterranean water are contributed to it from the surface of the earth by the water which filters downward, I am rather inclined to believe in a general diffusion of B. coli either on the surface of the earth, where it might be deposited with the dust of the air, or in the superficial layers of the earth, which may form one of its normal habitats." Therefore, the author considered that caution should be exercised in condemning a water on account of the presence of B. coli, except, as he added, "for those cases where it exists in considerable quantity."

Certain Italian observers appear to have come to even

less conservative conclusions. Abba (Abba, 1895) found colon bacilli constantly present in certain unpolluted waters near Turin. Moroni (Moroni, 1898; Moroni, 1899) reported the examination of numerous deep and shallow wells and unpolluted springs about Parma, as well as of the public water-supply of the city, for the colon bacillus and concluded that that organism was a water form and had no sanitary significance. The characters used for the identification of the species in this case were fairly exhaustive, but both Abba and Moroni used liter samples for analysis.

Levy and Bruns (Levy and Bruns, 1899) gave a new turn to the discussion by emphasizing the importance of animal inoculation, already suggested by Blachstein (Blachstein, 1893) and others. They claimed that the existence of numerous para-colon and para-typhoid organisms in air, in dust, and in unpolluted water made it impossible to decide by ordinary bacteriological methods whether true colon bacilli were present in water or not. In no case, however, did representatives of the colon group isolated by them from water kill a guinea-pig, even when 1 or 2 c.c. were injected intraperitoneally; and the authors, therefore, considered pathogenicity as an attribute belonging only to the true B. coli of the intestine. This paper aroused Professor Kruse's pupil, Weissenfeld, to a publication, in which the position of the Bonn school was carried to an extreme. Weissenfeld reported (Weissenfeld, 1900) the analysis of 30 samples of water supposedly pure, and of 26 samples considered to be contaminated. In

each case a single centimeter sample was first incubated in Parietti broth, and if no growth occurred, larger samples of half a liter or a liter were examined. Colon bacilli were found in all the samples examined; and the pathogenicity varied independently of the source of the water.[1] The author concluded that "the so-called Bacterium coli may be found in waters from any source, good or bad, if only a sufficiently large quantity of the water be taken for analysis." In the first place it should be noted that the characters used by this investigator for defining the "so-called Bacterium coli" were absolutely inadequate. He classed under that head all bacilli of medium size, which formed grapevine-leaf colonies on gelatin and gas in sugar agar, which were more or less motile, or rarely non-motile, and which were decolorized by the Gram method. As regards coagulation of milk and formation of indol, "the bacteria isolated differed." In the second place it is difficult to see how the author could possibly have believed that his experiments proved the isolation of the colon bacillus to be "useless as an aid in the sanitary examination of water," as the title of the paper runs. Even his own work furnishes strong evidence to the contrary. In 24 of the 26 samples from bad sources, he isolated his imperfectly defined colon bacilli from 1 c.c. of the water, while in only 8 of the 30 samples of good waters could he find such organisms in that quantity.

Of a series of 47 cultures of actic-acid bacteria

[1] Confirmed by Savage (Savage, 1903,.

recently examined by one of ourselves (Prescott, 1902 [a]; Prescott, 1903) 25 were found to give the reactions of B. coli. These organisms were isolated chiefly from cereals and products of milling, such as flour, bran, corn-meal, oats, barley, etc., while others were in technical use for producing the lactic fermentation. There is no evidence that any of these organisms were of intestinal origin, and yet they possess all the characters of typical colon bacilli, even to the pathogenic action when inoculated into guinea-pigs. These results explain the results of Klein and Houston (Klein and Houston, 1900), who reported the finding of typical colon bacilli in 3 out of 24 samples of wheat and oats obtained from a wholesale house; rice, flour, and oat-meal bought at two different retail shops gave B. coli on all three cereals in 1 case and on none in the other. In Germany, Papasotiriu (Papasotiriu, 1901) was meanwhile carrying on almost exactly similar investigations to Prescott's, with identical results.

What then does a colon test prove? Obviously (1) the presence of a colon bacillus which has come *directly* from the intestine of some animal, or (2) of a colon bacillus which has come indirectly from an animal, or (3) of a saprophytic "lactic-acid bacillus" which gives the same biochemical reactions. This immediately raises the interesting questions, Is it possible that the lactic-acid bacilli have been indirectly derived from animal intestines, having "escaped from cultivation," as the botanists say? Or is the converse true, namely, that all colon bacilli are simply lactic-acid bacteria which have found

in the warm intestinal canal richly supplied with food a favorable habitat?

The answer to these questions is of much theoretical interest, but need not be further considered here. The practical sanitary conclusions to be drawn are as follows:

1. Bacteria corresponding in every way to B. coli are by no means confined to animal intestines, but are widely distributed elsewhere in nature.

2. The finding of a few colon bacilli in large samples of water, or its occasional discovery in small samples, does not necessarily have any special significance.

3. The detection of B. coli in a large proportion of small samples (1 c.c. or less) examined is imperatively required as an indication of *recent* sewage pollution.

4. The *number* of colon bacilli in water rather than their *presence* should be used as a criterion of recent sewage pollution.

With these qualifications the value of the colon test was never more firmly established than it is to-day. Whether or not originally a domesticated form, it is clear that the colon bacillus finds in the intestine of the higher vertebrates an environment better suited to its growth and multiplication than any other which occurs in nature. It is almost certain that the only way in which large numbers of these organisms gain access to natural waters is by pollution with the domestic, industrial, and agricultural wastes of human life. If pollution has been recent, colon bacilli will be found in comparatively small samples of the waters. If pollution has been *remote* the

number of colon bacilli will be small, since there is good evidence that the majority of intestinal bacteria die from exposure in cold water. If derived from cereals or the intestines of wild animals the number will be insignificant except in the vicinity of great grain-fields or when the water receives refuse from grist-mills, tanneries, dairies, or lactic-acid factories.

The first recognition of the necessity for a quantitative estimation of colon bacilli in water we owe to Dr. Smith, who in 1892 (Smith, 1893[a]) outlined a plan for such a study to be made by the New York Board of Health on the Mohawk and Hudson Rivers. Burri (Burri, 1895) pointed out that the use of so large a sample as a liter for examination would lead to the condemnation of many good waters. Freudenreich (Freudenreich, 1895) at the same time indicated the necessity for taking into account the *number* of colon bacilli present. He recorded the isolation of the organism from unpolluted wells, when as large a quantity of water as 100 c.c. was used, and concluded that it was entirely absent only from waters of great purity and present in large numbers only in cases of high pollution. This author also quoted Miquel as having found colon bacilli in almost every sample of drinking-water if only a sufficient portion were taken for analysis, but gave no reference.

The practical results of the application of the colon test from this standpoint have always proved most instructive. As originally outlined by Dr. Smith, it consisted in the inoculation of a series of dextrose tubes with small

portions of water, tenths or hundredths of the cubic centimeter. It was first used by Brown (Brown, 1893) in 1892 for the New York State Board of Health, and its results showed from 22 to 92 fecal bacteria per c.c. in the water of the Hudson River at the Albany intake, and from 3 to 49 at various points in the Mohawk River between Amsterdam and Schenectady. In some previous work at St. Louis, the colon bacilli in the Mississippi River were found to vary from 3 to 7 per c.c.

Hammerl (Hammerl, 1897) used the presence of Bacillus coli as a criterion of self-purification in the river Mur. He considered, in spite of the position taken by Kruse, that when a water contained large numbers of colon bacilli, as well as an excess of bacteria in general, it might be considered to be contaminated by human or animal excrement. As, however, the organism would naturally be present in large quantities of such a water as that of the Mur, he used no enrichment process, but made plate cultures direct; he defined the B. coli as a small bacillus, non-motile or but feebly motile, growing rapidly at 37° C., coagulating milk and forming gas in sugar media. In general, Hammerl failed to find colon bacilli in the river by this method, except immediately below the various towns situated upon it; at these points of pollution he discovered a few colon colonies upon his plates, not more than 4 to 6 per c.c. of the water. He concluded that "the Bacterium coli, even when it is added to a stream in great numbers, under certain circumstances disappears very rapildy, so that it can no longer be detected

in the examination of small portions of the water." It should be noted that Hammerl's method was much less delicate than the use of the dextrose tube for preliminary incubation.

A very important series of observations carried out in England by the bacteriologists of the local government board has led to similar conclusions. Dr. Houston in particular (Houston, 1898; Houston, 1899[a]; Houston, 1900[a]) made an elaborate series of examinations of soils from various sources to see whether the microbes considered to be characteristic of sewage could gain access to water from surface washings free from human contamination. In the three papers published on this subject the examination of 46 soils was recorded. In only 10 of the samples was B. coli found, and of these 10, 9 were obviously polluted, being derived from sewage fields, freshly manured land, or the mud-banks of sewage-polluted rivers. The author finally concluded that "as a matter of actual observation, the *relative abundance* of B. coli in pure and impure substances is so amazingly different as to lead us to suspect that not only does B. coli not flourish in nature under ordinary conditions, but that it tends to even lose its vitality and die." "In brief, I am strongly of opinion that the presence of B. coli in any number, whether in soil or in water, implies *recent* pollution of animal sort." Pakes (Pakes, 1900) stated on the strength of an examination of "about 300 different samples of water," no particulars being published, that water from a deep well should not contain B. coli at all, but that water

from other sources need not be condemned unless the organism was found in 20 c.c. or less. When colon bacilli were found in only greater quantities than 100 c.c. the water might be considered as probably safe. Horrocks (Horrocks, 1901), after a general review of English practice, concluded that "when a water-supply has been *recently* polluted with sewage, even in a dilution of one in one hundred thousand, it is quite easy to isolate the B. coli from 1 c.c. of the water." "I would say that a water which contained B. coli so sparingly that 200 c.c. required to be tested in order to find it had probably been polluted with sewage, but the contamination was not of recent date." Chick (Chick, 1900) found 6100 colon bacilli per c.c. in the Manchester ship canal, 55–190 in the polluted River Severn, and numbers up to 65,000 per gram in roadside mud. On the other hand, of 38 unpolluted streams and rivulets, 31 gave no Bacillus coli and the other 7 gave 1 per c.c. or less. The Liverpool tap water, snow, rain, and hail gave no colon bacilli.

In the United States the colon test has been extensively applied during the last few years to certain polluted river-waters, in particular with the view of measuring the purification attained by sand filtration and that naturally occurring during the flow of a stream. A fairly good idea has thus been obtained of the numerical distribution of the B. coli in the larger rivers. Fuller (Fuller. 1899), for example, recorded the presence of colon bacilli in 60 per cent of the 1-c.c. samples taken from the Ohio at Cincinnati. When this water was passed through

either slow sand or mechanical filters, the effluent gave positive results about one-half the time in samples of 50 c.c.

The last three reports of the Massachusetts State Board of Health contain reports of the abundance of B. coli in a more highly polluted stream, the Merrimac at Lawrence. In 1898 (Massachusetts State Board of Health, 1899) the number of organisms found by making litmus-lactose-agar plates directly and inspecting the colonies varied from 20 per c.c. in May and June to 92 per c.c. in August and September, the average for the year being 47. Of 117 samples of the water which had passed through the city filter, only 9 showed the organism in a single colony.

In 1899 (Massachusetts State Board of Health, 1900) the study was considerably extended. The average number of colon bacilli in the river at the intake of the filter was again 47, and in only 1 sample out of 180 was it absent; below the city at the Lawrence Experiment Station the additional pollution raised the average number to 103. The effluent as it came directly from the filter showed B. coli in 24 per cent of the cubic centimeters examined, but at the outlet of the reservoir, the proportion had fallen to 7 per cent and at the Experiment Station, after passage through the distribution pipes, to only 4 per cent. The results obtained in the next year, 1900, were practically the same, but parallel tests were made in a larger volume of water by incubating with the addition of phenol

broth. The results of these comparative tests may be tabulated as follows:

PERCENTAGE OF SAMPLES OF WATER CONTAINING B. COLI.

LAWRENCE EXPERIMENT STATION (MASSACHUSETTS STATE BOARD OF HEALTH, 1901).

	Effluent of Filter.	Outlet of Reservoir.	Tap, City Hall.	Tap, Experiment Station.
In 1 c.c.	18.14	8.57	4.07	1.87
In 100 c.c.	38.12	23.30	15.54	15.54

It appeared that the use of the larger volume of water gave very little additional information, and indeed the real difference between the waters examined is rather obscured by the use of the large samples.

In 1900 Clark and Gage (Clark and Gage, 1900) reported some specially instructive observations made when certain of the underdrains of the Lawrence municipal water-filter were relaid in the autumn of 1898. In doing this work the sand on some of the beds was seriously disturbed; and in December, after the work was completed, B. coli was found in 1 c.c. of the filtered effluent in 72 per cent of the samples examined. In January and February the organisms were found in 54 per cent and 62 per cent of the samples, respectively, while in March the number fell to a normal value of 8 per cent. Corresponding to this excess of B. coli in the city water, there were 12 cases of typhoid fever in December, 59 cases in January, 12 in February, and 9 in March, all during the early part of the month. The authors conclude that "when filtering a river-water as polluted as that of the

Merrimac, it is safe to assume that when B. coli is found only infrequently in 1 c.c. of the effluent the typhoid germs, necessarily fewer in number and more easily removed by the filter, have been eliminated from the water."

Another interesting contribution to this question was made by the Massachusetts State Board of Health (Massachusetts State Board of Health, 1901) in connection with the examination of the spring-waters bottled for sale in the State. Ninety-nine springs were included in this study; and in almost every instance 4 samples were examined, 2 taken directly from the spring by the engineers of the board and 2 from the bottles as delivered for sale to the public. In the water of one spring B. coli was found twice, once in a sample from the spring and once in the bottled sample. This spring was situated in woodland, but was unprotected from surface drainage, and the method of filling bottles subjected it to possible contamination. In 5 other cases B. coli was found once in the sample from the spring; all were subject to pollution from dwellings or cultivated fields, and 4 of the 5 were shown to be highly contaminated, chemically. In 7 other cases B. coli was found in the bottled samples alone; 3 of these sources were of high purity, but the bottling process furnished opportunity for contamination.

Probably the most elaborate application of the colon test which has ever been attempted was made by Jordan in his recent examinations of the fate of the Chicago sewage in the Desplaines and Illinois Rivers. At one time

Professor Jordan was himself somewhat sceptical as to the value of the colon test, for he stated in 1890 (Jordan, 1890) that he had found, "in spring-water which was beyond any suspicion of contamination, bacteria which in form, size, growth on gelatin, potato, etc., were indistinguishable from B. coli commune." In his recent studies of self-purification (Jordan, 1901) the analyses were made quantitative by the examination of numerous measured samples, fractions of the cubic centimeter; and the method employed was the enrichment either in dextrose-broth fermentation-tubes or in phenol broth, with the subsequent making of litmus-lactose-agar plates. The cultures isolated on these plates were tested as to their behavior in dextrose broth, peptone solution, milk, and gelatin; of the dextrose tubes made directly from the water all were considered positive which gave more than 20 per cent gas in the closed arm, with an appreciable excess of hydrogen. The results were very significant. In fresh sewage a positive result was obtained about one-third of the time in one one-hundred-thousandth of a cubic centimeter and almost constantly in one ten-thousandth of a cubic centimeter. The Illinois and Michigan canal proved almost as bad, giving positive results on seven days out of twenty-eight in dilution of one in one hundred thousand and on twenty-eig[illegible] days out of thirty-two in a dilution of one in ten t[illegible] Morris, twenty-seven miles below Lockpo[illegible] canal enters the bed of the Desplaines Ri[illegible] miles below the entrance of the Kankakee,

diluting factor, the numbers were so reduced that positive results were obtained only on eleven days out of twenty in one-thousandth of a cubic centimeter, on twenty days out of thirty in one-hundredth of a cubic centimeter, and on twenty days out of twenty-three in one-tenth of a cubic centimeter. At Averyville, one hundred and fifty-nine miles below Chicago, colon bacilli were isolated on only four days out of twenty-seven in one-tenth of a cubic centimeter, and on thirteen days out of thirty-one in one cubic centimeter. A comparison with certain neighboring rivers showed this to be about the normal value for waters of that character, as the following table extracted from Professor Jordan's paper will show.

NUMBER OF B. COLI PRESENT IN CERTAIN RIVER WATERS.

(Jordan, 1901.)

Source of Sample.	.1 c.c. No. Days Water Examined.	.1 c.c. No. Days B. Coli Found.	1 c.c. No. Days Water Examined.	1 c.c. No. Day B. Coli Found.
Illinois River, Averyville....	27	4	31	13
Mississippi River, Grafton..	34	10	35	23
Fox River.....	22	2	23	6
Sangamon River.....	25	14	27	21
Missouri River.....	32	13	31	21

These results harmonize rather closely with those previously recorded by Brown and Fuller and indicate that in the larger rivers where the proportionate pollution is not extreme, colon bacilli may be isolated in about half the 1-c.c. samples examined. Such rivers are of course inadmissible as sources of water-supply, according to modern sanitary standards, unless subjected to preliminary treatment.

More recently Hunnewell and one of ourselves (Winslow and Hunnewell, 1902 [b]) examined a considerable series of normal waters for B. coli, testing 1 c.c. from each by the dextrose-broth method and a larger portion of 100 c.c. by incubation with phenol broth as described in Chapter VI. The samples were obtained from the public supplies of Taunton, Boston, Cambridge, Braintree, Brookline, Needham, and Lynn in Massachusetts, and of Newport, R. I., from the Sudbury River, from the ocean, from the waters of springs bottled for the market, from ponds, pools of rain and melted snow, springs, brooks, shallow wells, and driven wells in various towns near the city of Boston. For comparison 50 samples of polluted waters from the Charles, Mystic, Neponset, and North Rivers were examined. The colon bacillus was defined as outlined in Chapter VI; and organisms which lacked the power to reduce nitrates or to form indol were classed in the "Paracolon group." The results are summarized in the following table:

PRESENCE OF B. COLI IN POLLUTED AND UNPOLLUTED WATERS.

(Winslow and Hunnewell, 1902[b].)

UNPOLLUTED WATERS.

	1 c.c.	100 c.c.
Samples examined	157	153
Dextrose broth positive	40	76
Lactose plate positive	13	31
Colon group	5	11
Paracolon group	5	5
B. cloacæ group	...	5
Streptococcus group	3	10

POLLUTED WATERS.

	1 c.c.	100 c.c.
Samples examined	50	48
Dextrose broth positive	50	37
Lactose plate positive	50	26
Colon group	18	4
Paracolon group	6	
Streptococcus group	25	22
B. cloacæ	1	

The authors pointed out that these tables indicate that bacteria capable of growth at the body temperature and fermenting dextrose and lactose are infrequently found in unpolluted waters, and colon bacilli are very rarely present, since in 157 samples typical colon bacilli only appeared 5 times. In the polluted waters it is evident that colon bacilli originally present were in many cases killed out during the process of enrichment by the streptococci, since every one of the dextrose-broth tubes showed gas at the first incubation of 1 c.c.

The latest important contribution to this subject comes from Dantzic. Petruschky and Pusch (Petruschky and Pusch, 1903) examined a considerable series of waters from different sources by incubating measured samples with equal amounts of nutrient broth and isolating upon agar. In 45 samples of well-waters they found B. coli 7 times in .01 c.c., 9 times in .1 c.c., and 7 times in 1 c.c. In the other 22 cases it could not be found in 1 c.c. and in 4 cases not in 100 c.c. One sample showed it only in .600 c.c. and 1 not in 750 c.c. Of 29 river-waters, only 2 failed to give positive results in .1 c.c. and 14 showed B. coli in .001 of a c.c. or less. In sewage the number varied from

1 to 1,000,000 per c.c. The authors conclude that a quantitative estimation of the B. coli content furnishes a good measure of the fecal pollution of water and that the observed differences in the extent of B. coli contamination of surface-waters are so great that they differ from each other more than a million-fold.

Altogether the evidence is quite conclusive that the absence of B. coli demonstrates the harmlessness of a water as far as bacteriology can prove it. That when present, its numbers form a reasonably close index of the amount of pollution the authors above quoted have proved beyond reasonable cavil. It may safely be said that when the colon bacillus, as defined by the tests above, is found in such abundance as to be isolated in a large proportion of cases from 1 c.c. of water, it is reasonable proof of the presence of serious pollution, a view which is to-day accepted by most American bacteriologists.

CHAPTER VIII.

PRESUMPTIVE TESTS FOR B. COLI.

THE isolation and identification of B. coli by the methods which have been described is a time-consuming and laborious operation, and one sometimes impossible to apply in the practical supervision of a water-supply.

Hence it is of especial value to the engineer to have certain tests which may be easily and quickly carried out and which will give a probably correct idea as to whether pollution does or does not exist. Such tests are spoken of as "presumptive tests." If positive, the water showing such a test may be regarded as suspicious, but must be further examined to *prove* the presence of fecal bacteria. If the presumptive test is negative, no further examination need be made. Of the cultural reactions required to establish the identity of the colon bacillus, the growth and gas formation in dextrose broth, as originally suggested by Smith (1893[b]) is so well marked and so strongly typical of B. coli that this medium may be most conveniently used as a "presumptive test."

The details of the operation vary in individual laboratories, but the underlying principle in all is that B. coli develops rapidly in dextrose broth with gas formation of

from 25 to 70 per cent of the capacity of the closed arm of the fermentation-tube. Of this gas approximately one-third is carbon dioxide and two-thirds hydrogen, that is, as the gas formula is generally expressed, $\frac{H.}{CO_2} = \frac{2}{1}$.

In testing a water by this method a series of samples of the water, in suitable dilution, .001, .01, .1, 1.0, or 10 c.c., is added directly to the dextrose-broth tubes and incubated for twenty-four hours at 37°.

On measurement of the gas, if the results above given are obtained, the reaction is considered typical. If the amount of gas is between 10 and 25 per cent or more than 70 per cent, or if the percentage of carbon dioxide is greater than 40, the reaction is considered atypical. If no gas forms, or less than 10 per cent, the test is called negative. It is recognized that the distinction is not absolute, since even pure cultures of B. coli sometimes fail to give typical reactions, and on the other hand, an atypical organism sometimes gives a satisfactory positive test. There can be little question, however, as to its value to the sanitary engineer.

In recent years, Irons (Irons, 1901) was perhaps the first to call attention to the value of this method, stating that "when the dextrose tube yields approximately 33 per cent of CO_2 Bacillus coli communis is almost invariably present." In the next year the reliability of the fermentation test as an indication of B. coli was worked out by Gage (Gage, 1902) as given in the following table.

	1 c.c.	100 c.c.
Number of samples tested	5172	1375
" giving preliminary fermentation	1036	474
Per cent of latter proved to contain coli	70	71

The work of Hunnewell and one of ourselves (Winslow and Hunnewell, 1902[b]) showed that while 50 samples of polluted waters all gave gas in the dextrose tube, only 40 out of 157 samples from presumably unpolluted sources showed a similar fermentation. Whipple (Whipple, 1903) examined a large number of surface-water supplies by the "presumptive test" and obtained striking results, shown in the following table. The waters are arranged in six groups according to the results of sanitary inspection, group I including waters collected from almost uninhabitated watersheds and group VI waters too much polluted to be safely used for domestic purposes.

PERCENTAGE OF SAMPLES OF WATERS OF VARIOUS SANITARY GRADES GIVING POSITIVE TESTS FOR B. COLI WHEN DIFFERENT AMOUNTS WERE EXAMINED.

(Whipple, 1903.)

Group.	0.1 c.c.	1.0 c.c.	10 c.c.	100 c.c.	500 c.c.
I	0.0	3.5	20.8	50.0	50.0
II	5.0	7.3	15.0	60.0	60.0
III	0.0	7.0	50.0	50.0	60.0
IV	4.0	6.8	41.7	67.0	75.0
V	5.0	13.0	75.0	100.0	100.0
VI	5.0	20.2	75.0	80.0	100.0

In view of these results Whipple suggests the following provisional scheme of interpretation, which seems to the authors to be, in general, sound.

Sanitary Quality.	Presumptive Test for Bacillus Coli.				
	0.01 c.c.	0.1 c.c.	1.0 c.c.	10.0 c.c.	100 c.c.
Safe	o	o	o	o	+
Reasonably safe	o	o	o	+	+
Questionable	o	o	+	+	+
Probably unsafe	o	+	+	+	+
Unsafe	+	+	+	+	+

Results harmonizing well with the above were obtained by Nibecker and one of us (Winslow and Nibecker, 1903), as quoted in Chapter IV. Of 775 dextrose tubes inoculated from 259 samples of water from apparently unpolluted sources, only 41 showed gas and only 3 gave the gas formula characteristic of B. coli.

The litmus-lactose-agar plate furnishes another presumptive test of considerable value as indicated in Chapter IV, although it is probably less delicate than the dextrose-broth method. With polluted waters in particular this test will be found of value, since streptococci may interfere with the dextrose-broth method if dilution is insufficient or incubation too prolonged.

Certain special media have also been suggested for rapid routine water analysis of which those containing "neutral red," one of the safranine dyes, has been most fully studied. Rothberger (Rothberger, 1898) first pointed out that B. coli reduces solutions of this substance, the color changing to canary-yellow accompanied by green fluorescence. Makgill (Makgill, 1901), Savage (Savage, 1901), and other English observers report favorable

results from the use of this test, but according to American standards, Irons (Irons, 1902) and Gage and Phelps (Gage and Phelps, 1903) conclude that the group of organisms giving a positive neutral red reaction is too large a one to give very valuable sanitary information. In a paper read at the Washington meeting of the American Public Health Association in 1903, Stokes suggested a modification of this medium in which lactose is substituted for dextrose, since many saprophytic organisms will attack the latter and not the former sugar.

The medium devised by MacConkey (MacConkey, 1900; MacConkey, 1901; MacConkey and Hill, 1901) containing sodium taurocholate as its characteristic ingredient has also proved successful in the hands of some observers. The dextrose fermentation-tube, however, is the only presumptive test whose value has been established by conclusive general investigations.

CHAPTER IX.

OTHER INTESTINAL BACTERIA.

It would be an obvious advantage if the evidence of sewage contamination, furnished by the presence of B. coli could be reinforced or confirmed by the discovery in water of other forms equally characteristic of the intestinal canal. The attention of a few bacteriologists in England and America has been turned strongly in this direction during the past few years; and two groups of organisms, the sewage streptococci and the anærobic spore-bearing bacilli, have been described as being probably significant.

The term "sewage streptococci" covers an ill-defined group including many organisms which do not actually occur in well-marked chains. Those most commonly found correspond, however, rather closely to the type of Str. erysipelatos. They grow feebly on the surface of ordinary nutrient agar, producing faint transparent, rounded colonies, but under semi-anærobic conditions flourish better, giving a well-marked growth along the gelatin stab and only a small circumscribed film on the surface. They are favored by the presence of the sugars and ferment dextrose and lactose, with the formation of abundant acid but no gas. They are seen under the

287035

microscope as cocci, occurring as a rule in pairs, short chains, or irregular groups. They do not show visible growth in the standard peptone and nitrate solutions; most of them do not liquefy gelatin, though occasionally forms are found which possess this power. No systematic study of the various species found in the intestine has so far been made, and at present all cocci giving the characteristic growth on agar and strongly fermenting lactose may be included as "sewage streptococci."

Although the significance of the streptococci as sewage organisms is not established with the same definiteness which marks our knowledge of the colon group, these organisms have been isolated so frequently and with such constant results that it now seems reasonable to regard their presence as indicative of pollution. Although originally reported by Laws and Andrewes (Laws and Andrewes, 1894), their importance was not emphasized until 1899 and 1900, when Houston (Houston, 1899[b], 1900[b]) laid special stress upon the fact that streptococci and staphylococci seem to be characteristic of sewage and animal waste, the former being, in his opinion, the more truly indicative of dangerous pollution, since they are "readily demonstrable in waters recently polluted and seemingly altogether absent from waters above suspicion of contamination." In the water of 6 rivers recently extensively sewage-polluted he found streptococci in from one-tenth to one ten-thousandth of a c.c. of the water examined, although in some cases the chemical analysis did not clearly indicate dangerous pollution. On the

other hand, 8 rivers, polluted, but not recently and extensively polluted, showed no streptococci in one-tenth of a c.c., although the chemical and the ordinary bacteriological tests gave results which would condemn the waters. Horrocks (Horrocks, 1901) found these organisms in great abundance in sewage and in waters known to be sewage-polluted, but which contained no traces of Bacillus coli. He found by experiment that B. coli gradually disappeared from many specimens of sewage kept in the dark at the temperature of an outside veranda, while the commonest forms which persisted were varieties of streptococci and staphylococci.

In America attention was first called to these organisms by Hunnewell and one of ourselves (Winslow and Hunnewell, 1902[a]), and the same authors later (Winslow and Hunnewell, 1902[b]) recorded the isolation of streptococci from 25 out of 50 samples of polluted waters. Gage (Gage, 1902), from the Lawrence Experiment Station has reported the organisms present in the sewage of that city, while one of us (Prescott, 1902[b]) has shown that they are abundant in fecal matter and often overgrow B. coli in a few hours when inoculations are made from such material into dextrose broth. In the recent monograph of Le Gros (Le Gros, 1902) of the many streptococci described all without exception were isolated, either from the body or from sewage. In the study of 259 samples of presumably unpolluted waters, by the method of direct plating, Nibecker and one of the authors (Winslow and Nibecker, 1903) found streptococci in

only 1 sample, while Baker and one of us (Prescott and Baker, 1904) found the organism present in each of 50 samples of variously polluted waters when inoculation was first made into dextrose broth from which litmus-lactose-agar plates were made after various intervals.

The isolation of these organisms either from plates or liquid cultures is easy. On the lactose-agar plate, made directly from a polluted water the colonies of the streptococci may generally be distinguished from those of other acid-formers by their small size, compact structure, and deep-red color, which is permanent, never changing to blue at a later period of incubation as the colonies of B. coli may do. Developing somewhat slowly, however, they may be overlooked if present only in small numbers. In the dextrose-broth tube, streptococci will appear in abundance after a suitable period of incubation. Prescott and Baker in the work above mentioned found that with mixtures of B. coli and streptococci in which the initial ratios of the latter to the former varied from 1:94 to 208:1, the colon bacilli developed rapidly during the early part of the experiment, reaching a maximum after about fourteen hours and then diminishing rapidly, while the streptococci first became apparent after ten to fifteen hours and reached their maximum after twenty to sixty hours, according to the number originally present.

Applying the same method to polluted waters, similar periodic changes were observed; pure cultures of B. coli were first obtained, then the gradual displacement of one form by the other took place, and at length streptococci

were either present in pure culture or in great predominance as shown by the accompanying tables. The samples of water were plated directly upon litmus-lactose-agar and he plates were incubated at 37° for twenty-four hours, when the red colonies were counted. At the time of plating, 1 c.c. from each sample was also inoculated into dextrose broth in fermentation-tubes, which were likewise incubated at 37°. After various periods, as indicated by the tables below, the tubes were shaken thoroughly and 1 c.c. of the contents withdrawn. This was then diluted (generally 1–1,000,000) with sterile water, plated with

TABLE I.

RELATIVE GROWTH OF B. COLI AND SEWAGE STREPTOCOCCI FROM POLLUTED WATERS IN DEXTROSE BROTH.

(Prescott and Baker, 1904.)

Sample Number			1	2	3	4	5	6	7	8	9	10
Red colonies developing from 1 c.c. of original sample on litmus-lactose-agar			4	10	9	5	8	55	35	460	1250	105
Number found, in millions per cubic centimeter, after growth in dextrose broth for various periods	11 hrs.	B. coli	0	20	68.4	200	185	400	130	332	420	410
		Strept.	0	0	0	0	0	0	0	0	0	0
	16 hrs.	B. coli	200	76	130	270	220	210	140	420	285	410
		Strept.	40	25	20	10	45	30	20	210	75	145
	23 hrs.	B. coli	280	150	385	370	300	570	200	405	320	300
		Strept.	140	85	280	170	300	1700	110	350	370	350
	39 hrs.	B. coli	0	0	25	110	0	210	20	24	105	
		Strept.	474	420	480	300	390	170	400	105	250	
	63 hrs.	B. coli	0	0	0	0	0	12	8	0	0	0
		Strept.	2	0	0	45	1	2	45	150	86	170
First gas noted after (hrs)			10	10	9	9	10	8	10	6	6	8

TABLE II.

RELATIVE GROWTH OF B. COLI AND SEWAGE STREPTOCOCCI FROM POLLUTED WATERS IN DEXTROSE BROTH.

(Prescott and Baker, 1904.)

Sample Number			18	19	20	21	22	23	24	25
Red colonies developing from 1 c.c. of original sample on litmus-lactose-agar			1	150	25	30	50	170	200	30
Number found, in millions per cubic centimeter, after growth in dextrose broth for various periods	7 hrs.	B. coli	.02	....	....	.01	.04	.12	.55	1.6
		Strept.	0	....	....	0	0	0	0	0
	17 hrs.	B. coli	266	100	88	350	510	380	330	160
		Strept.	150	0	40	140	240	128	80	220
	27 hrs.	B. coli	520	610	72	700	1000	740	100	300
		Strept.	800	860	670	1080	2500	4380	....	3900
	40 hrs.	B. coli	0	0	10	22	36	7	7	
		Strept.	252	330	260	22	66	60	52	
	52 hrs.	B. coli	10	16	38	20	70	35	10	27
		Strept.	40	16	3.8	31	41	25	10	30

litmus-lactose-agar in the usual way, and incubated for twenty-four hours. The colonies of B. coli and streptococci were distinguished microscopically, and by difference in color and general characters.

These facts suggest the following method for the detection of B. coli and sewage streptococci in polluted waters.

Inoculate the desired quantity of water, preferably 1 c.c., into dextrose broth, in a fermentation-tube, and incubate at 37°. After a few hours' incubation examine the cultures for gas. Within two or three hours after gas formation is first evident, plate from the broth in litmus-lactose-agar, incubating for twelve to eighteen hours at

37°. If at the end of this time no acid-producing colonies are found, it is probably safe to assume that there were no colon bacilli present. On the other hand, if red colonies are developed, these must be further examined by the regular diagnostic tests for B. coli. After the first plating from the dextrose broth, replace the fermentation-tube in the incubator and allow it to remain for twenty-four to thirty-six hours, then plate again in litmus-lactose-agar. This plating should give a nearly pure culture of streptococci if these organisms were originally present in the water. If colon bacilli are not found in the first set of plates the streptococci may still be isolated by this method.

The relative relation of the streptococci and the colon bacilli to sewage pollution is still uncertain. Houston (Houston, 1900) held that the former microbes imply "animal pollution of extremely recent and therefore specially dangerous kind." Horrocks (Horrocks, 1901), on the other hand, maintains, largely on the strength of certain experiments with stored sewage, that the streptococci persist after colon bacilli have disappeared and indicate contamination with old sewage which is not necessarily dangerous. On the whole, it seems probable that the streptococci, like the colon bacilli, are primarily parasitic organisms, the former being associated both with the outer and inner surfaces of the body. Like the colon bacilli, their presence in water indicates contact with the wastes of human life, and their isolation from a suspected sample furnishes valuable confirmatory evidence of its dangerous character.

The English bacteriologists have ascribed much importance as indicators of sewage pollution to another group of organisms, the anærobic spore-forming bacilli, of which the B. sporogenes is a type. This form was isolated by Klein (Klein, 1898; Klein, 1899) in 1895, in the course of an epidemic of diarrhœa at St. Bartholomew's Hospital, and described under the name of B. enteritidis sporogenes; it is apparently identical with the B. aerogenes capsulatus of Welch (Welch and Nuttall, 1892).

Klein's procedure for isolating the B. sporogenes is simple in the case of polluted waters. A portion of the sample to be examined is added to a tube of sterile milk, which is then heated to 80° C. for ten minutes to destroy vegetative cells. The milk is then cooled and incubated under anærobic conditions, which may be accomplished most conveniently by Wright's method. A tight plug of cotton is forced a quarter way down the test-tube, the space above is loosely filled with pyrogallic acid, a few drops of a strong solution of caustic potash are added, and the tube is tightly closed with a rubber stopper. After eighteen to thirty-six hours at 37° the appearance of the tube will be very characteristic if the B. sporogenes is present. "The cream is torn or altogether dissociated by the development of gas, so that the surface of the medium is covered with stringy, pinkish-white masses of coagulated casein, enclosing a number of gas-bubbles. The main portion of the tube formerly occupied by the milk now contains a colorless, thin, watery whey, with a few casein lumps adhering here and there to the sides of

the tube. When the tube is opened, the whey has a smell of butyric acid and is acid in reaction. Under the microscope the whey is found to contain numerous rods, some motile, others motionless."

The B. sporogenes when isolated in pure culture on glucose agar is a stout rod. It liquefies gelatin, forming in this medium large oval spores. It is strongly pathogenic for guinea-pigs, by which character it is distinguished from the B. butyricus of Botkin.

The researches of Klein and Houston (Klein and Houston, 1898, 1899) have shown that the B. sporogenes occurs in English sewage in numbers varying from 30 to 2000 per c.c. and that it is often absent in considerable volumes of pure water. In Boston sewage it may usually be isolated from .01 or .001 of a c.c. (Winslow and Belcher, 1904).

Evidently in order to have any significance, an examination for this organism must be made with large samples and the concentration of at least 2000 c.c. of water through a Pasteur filter is recommended by Horrocks as a necessary prelude (Horrocks, 1901). Since the spores of an anærobic bacillus may persist for an indefinite period in polluted waters, their presence need not necessarily indicate recent or dangerous pollution. Since the number present even in sewage is so small and so variable, no quantitative standard can be established; on the whole, it does not appear that the practical application of the anærobic test will ever be a wide one.

There are numerous other sewage bacteria whose presence is more or less constantly characteristic of pol-

luted waters. Organisms of the Proteus group are sometimes present, exhibiting marked morphological variations, from the coccus form to long twisted threads and forming on gelatin irregular amœboid colonies with filiform processes extending into the surrounding gelatin. The B. subtilis group of strongly ærobic spore-forming bacilli, giving a brown wrinkled parchment-like growth on agar, and moss-like liquefying colonies on gelatin, is usually represented. Among the allies of B. coli may be mentioned B. ærogenes, which differs from it in being non-motile, failing to produce indol, and forming spherical drop-like colonies on gelatin. B. cloacæ resembles B. coli in most respects, but causes a liquefaction of gelatin. The property of liquefaction was formerly believed to be of general significance, inasmuch as the liquefying bacteria were regarded as closely allied to intestinal organisms, and in themselves indicative of pollution. This position is, however, no longer tenable, since many bacteria, typical of the purest waters, may cause liquefaction.

While the organisms above mentioned, and many others as well, deserve notice in the examination of gelatin plates from a suspected water, none of them is of sufficient importance to warrant any special procedure for their isolation.

CHAPTER X.

THE SIGNIFICANCE AND APPLICABILITY OF THE BACTERIOLOGICAL EXAMINATION.

THE first attempt of the expert called in to pronounce upon the character of a potable water should be to make a thorough sanitary inspection of the pond, stream, or well from which it is derived. Study of the possible sources of pollution on a watershed, of the direction and velocity of currents above and below ground, of the character of soil and the liability to contamination by surface-wash are conceded to yield evidence of the greatest value. Often, however, some opinion must be formed upon the quality of water sent from a distance without the opportunity of examining its surroundings; and even when sanitary inspection can be made, its results are by no means conclusive. No reconnoissance can show certainly whether unpurified drainage from a cesspool does or does not reach a given well; whether sewage discharged into a lake does or does not find its way to a neighboring intake; whether pollution of a stream has or has not been removed by a certain period of flow. Evidence upon these points must be obtained from a careful study of the

characteristics of the water in question, and this study can be carried out along two lines, chemical and bacteriological.

A chemical examination of water for sanitary purposes, is mainly useful in throwing light upon one point—the amount of decomposing organic matter present. Humus-like substances may be abundant in surface-waters quite free from harmful pollution, but these are stable compounds. Easily decomposable bodies, on the other hand, must obviously have been recently introduced into the water and mark a transitional state. "The state of change is the state of danger," as Dr. T. M. Drown has phrased it. Sometimes the organic matter has been washed in by rain from the surface of the ground, sometimes it has been introduced in the more concentrated form of sewage. In any case, it is a warning of possible pollution, and the determination of free ammonia, nitrites, carbonaceous matter, as shown by "oxygen consumed," and dissolved oxygen yield important evidence as to the sanitary quality of a water.

Furthermore, nitrates, the final products of the oxidation of organic matter, and the chlorine introduced as common salt into all water which has been in contact with the wastes of human life, furnish additional information as to the antecedents of a sample. The results of the chlorine determination are indeed perhaps more clear than those of any other sanitary analysis, for chlorine and sewage pollution vary together, due allowance being made for the proximity of the sea and other geological

and meteorological factors. Unfortunately, it is only past history and not present conditions which these latter, tests reveal, for in a ground-water completely purified from a sanitary standpoint such soluble constituents remain, of course, unchanged. Thus, in the last resort, it is upon the presence and amount of decomposing organic matter in the water studied that the opinion of the chemist must be based.

The decomposition of organic matter may be measured either by the material decomposed or by the number of organisms engaged in carrying out the process of decomposition. The latter method has the advantage of far greater delicacy, since the bacteria respond by enormous multiplication to very slight increase in their food-supply, and thus it comes about that the standard gelatin-plate count at 20° roughly corresponds, in not too heavily polluted waters, to the free ammonia and "oxygen consumed," as revealed by chemical analysis. If low numbers of bacteria are found, the evidence is highly reassuring, for it is seldom that water could be contaminated under natural conditions without the direct addition of foreign bacteria or of organic matter which would condition a rapid multiplication of those already present. The bacteriologist in such cases can declare the innocence of the water with justifiable certainty. When high numbers are found the interpretation is less simple, since they may exceptionally be due to the multiplication of certain peculiar water forms. Large counts, however, under ordinary conditions, when including a normal variety of

forms indicate the presence of an excess of organic matter derived in all probability either from sewage or from the fresh washings of the surface of the ground. In either case danger is indicated.

A still closer measure of polluting material may be obtained from the numbers of colonies which develop on litmus-lactose-agar at 37°, since organisms which thrive at the body temperature, and particularly those which ferment lactose, are characteristic of the intestinal tract and but rarely occur in normal waters.

Finally, the search for the Bacillus coli furnishes the most satisfactory of all single tests for fecal contamination. This organism is pre-eminently a denizen of the alimentary canal and may be isolated with ease from waters to which even a small proportion of sewage has been added. On the other hand, it is never found in abundance in waters of good sanitary quality; and its numbers form an excellent index of the value of waters of an intermediate grade. The streptococci appear to be forms of a similar significance useful as yielding a certain amount of confirmatory evidence. The full bacteriological analysis should then consist of three parts—the gelatin-plate count, as an estimate of the amount of organic decomposition in process; the total count, and the count of red colonies, on litmus-lactose-agar, as a measure of the organisms which form acids and thrive at the body temperature; and the study of a series of dextrose-broth tubes for the isolation of colon bacilli and streptococci. The simple examination of the dextrose-broth tube and the count on

the litmus-lactose-agar plate serve for what Whipple has well called presumptive tests.

The results of the bacteriological examination have, in several respects, a peculiar and unique significance. First, this examination is the most *direct* method of sanitary water analysis. What we dread in drinking-water is the presence of pathogenic bacteria, mainly from the intestinal tract of man, and it is quite certain that the related non-pathogenic bacteria from the same source will behave more nearly as these disease germs do than will any chemical compounds. In the second place, the bacteriological methods are superior in *delicacy* to any others. Klein and Houston (Klein and Houston, 1898) showed by experiment with dilutions of sewage that the colon test was from ten to one hundred times as sensitive as the methods of chemical analysis; and studies of the self-purification of streams have confirmed his results on a practical scale. Thus in the Sudbury River it was found that while the chemical evidences of pollution persisted for six miles beyond the point of entrance, the bacteria introduced could be detected for four miles further (Woodman, Winslow, and Hansen, 1902).

The statement is sometimes made that while bacteriological methods may be more delicate for the detection of pollution in surface-waters, contamination in ground-waters may best be discovered by the chemical analysis. That such is not the case has been well shown by Whipple (Whipple, 1903), who cites the following

two instances in which the presumptive test revealed contamination not shown by the chemical analysis:

"A certain driven-well station was located in swampy land along the shores of a stream, and the tops of the wells were so placed that they were occasionally flooded at times of high water. The water in the stream was objectionable from the sanitary standpoint. The wells themselves were more than 100 feet deep; they penetrated a clay bed and yielded what may be termed artesian water. Tests for the presence of Bacillus coli had invariably given negative results, as might be naturally expected. Suddenly, however, the tests became positive and so continued for several days. On investigation it was found that some of the wells had been taken up to be cleaned, and that the workmen in resinking them had used the water of the brook for washing them down. This allowed some of the brook-water to enter the system. It was also found that at the same time the water in the brook had been high, and because of the lack of packing in certain joints at the top of the wells the brook-water leaked into the suction main. The remedy was obvious and was immediately applied, after which the tests for Bacillus coli once more became negative. During all this time the chemical analysis of the water was not sufficiently abnormal to attract attention. On another occasion a water-supply taken from a small pond fed by springs, and which was practically a large open well, began to give positive tests for Bacillus coli, and on examination it was found that a gate which kept out the water

of a brook which had been formerly connected with the pond was open at the bottom, although it was supposed to have been shut, thus admitting a contaminated surface-water to the supply." Whipple also calls attention to the report on the Chemical and Bacteriological Examination of Chichester Well-waters by Houston (Houston, 1901), in which the results of chemical and bacteriological examinations of 30 wells were compared. It was found that the bacteriological results were in general concordant and satisfactory. The wells which were highest in the number of bacteria showed also the greatest amount of pollution, as indicated by the numbers of B. coli, B. sporogenes, and streptococci. On the other hand, the chlorine and the albuminoid ammonia showed no correspondence with the bacteriological results.

Thirdly, negative tests for Bacillus coli and low bacterial counts may be interpreted as proofs of the good quality of water, with a *certainty* not attainable by any other method of analysis. Many a surface-water with reasonably low chlorine and ammonias has caused epidemics of typhoid fever; but it is impossible under any natural conditions that a water could contain the typhoid bacillus without giving clear evidence of pollution in the dextrose-broth tube or on the lactose-agar plate.

It seems to the writers that the real application of chemistry begins where that of bacteriology ends. When pollution is so gross that its existence is obvious and only its amount needs to be determined, the bacteriological tests will not serve, on account of their excessive delicacy.

In studying the heavy pollution of small streams, the treatment of trades wastes, and the purification of sewage, the relations of nitrogeneous compounds and of oxygen compounds are of prime importance. In other words, when pollution is to be avoided, because the decomposition of chemical substances causes a nuisance, it must be studied by chemical methods. When the danger is sanitary and comes only from the presence of bacteria, bacteriological methods furnish the true index of pollution.

In the study of certain special problems the paramount importance of bacteriology is generally recognized. The distribution of sewage in large bodies of water into which it has been discharged may thus best be traced on account of the ready response of the bacterial counts to slight proportions of sewage, particularly since the ease and rapidity with which the technique of plating can be carried out make it possible to examine a large series of samples with a minimum of time and trouble. The course of the sewage carried out by the tide from the outlet of the South Metropolitan District of Boston was studied in this way by E. P. Osgood in 1897, and mapped out by its high bacterial content with greater accuracy than could be attained by any other method. Some very remarkable facts have been developed by similar studies as to the persistence of separate streams of water in immediate contact with each other. Heider showed that the sewage of Vienna, after its discharge into the Danube River, flowed along the right bank of the stream, preserving its own bacterial characteristics, and

not mixing perfectly with the water of the river for a distance of more than twenty-four miles (Heider, 1893). Jordan (Jordan, 1900), in studying the self-purification of the sewage discharged from the great Chicago drainage canal, found by bacteriological analyses that the Des Plaines and the Kankakee Rivers could both be distinguished flowing along in the bed of the Illinois, the two streams being in contact, yet each maintaining its own individuality. Finally, the quickness with which slight changes in the character of a water are marked by fluctuations in bacterial numbers renders the bacteriological methods invaluable for the daily supervision of surface supplies or of the effluents from municipal filtration plants.

In the commoner case, when normal values obtained by such routine analyses are not at hand, the problem of the interpretation of any sanitary analysis is a more difficult one. The conditions which surround a source of water-supply may be constantly changing. No engineer can measure the flow of a stream in July and deduce the amount of water which will pass in February; yet the July gauging has its own value and significance. So a single analysis of any sort is not sufficient for all past and future time. If it gives a correct picture of the hygienic condition of the water at the moment of examination it has fulfilled its task, and this the bacteriological analysis can do. The evidence furnished by inspection and by chemical analysis should be sought for and welcomed whenever it can be obtained, yet we are of the opinion

that, on account of their directness, their delicacy, and their certainty, the bacteriological methods should least of all be omitted, and, if necessary, they alone may furnish conclusive testimony as to the safety of a potable water.

APPENDIX.

It has been pointed out that the number of bacteria developing from a given sample of water will be largely dependent upon the composition and character of the culture medium. As the nature of the medium varies widely according to the method of preparation, workers in different laboratories can have no rational basis for comparison of results until their methods are essentially uniform.

To make results comparable as far as possible, the Laboratory Section of the American Public Health Association has adopted standard methods for the preparation of the commonly used nutrient media given in the following extract from the report of the Committee (Fuller, 1902).

STANDARD METHODS FOR GELATIN AND AGAR.

	GELATIN.	AGAR.
1.		Boil 15 gm. thread agar in 500 c.c. water for half an hour and make up weight to 500 gm. or digest for 10 minutes in the autoclav at 110° C. Let this cool to about 60° C.
2.	Infuse 500 gm. lean meat 24 hours with 1000 c.c. of distilled water in refrigerator.	Infuse 500 gm. lean meat 24 hours with 500 c.c. of distilled water in refrigerator.
3.	Make up any loss by evaporation.	
4.	Strain infusion through cotton flannel.	
5.	Weigh filtered infusion.	

	GELATIN.	AGAR.
6.	Add 1% Witte's peptone and 10% gold label sheet gelatin.	Add 2% of Witte's peptone.
7.	Warm on water-bath, stirring till peptone and gelatin are dissolved and not allowing the temperature to rise above 60° C.	
8.		Neutralize.
9.		To 500 gm. of the meat infusion add 500 c c. of the 3% agar, keeping the temperature below 60° C.
10.	Heat over boiling water (or steam) bath 30 minutes.	
11.	Restore loss by evaporation.	
12.	Titrate, after boiling 1 minute to expel carbonic acid.	
13.	Adjust reaction to +1.0% by adding normal hydrochloric acid or sodium hydrate as required.	
14.	Boil 2 minutes over free flame, constantly stirring.	
15.	Make up loss by evaporation.	
16.	Filter through absorbent cotton and cotton flannel, passing the filtrate through the filter until clear.	
17.	Titrate and record the final reaction.	
18.	Tube, using 5 c.c. in each tube in the case of gelatin, and 7 c.c. in the case of agar.	
19.	Sterilize 15 minutes in the autoclav at 110°, or for 30 minutes in streaming steam on three successive days.	
20.	Store in the ice-chest in a moist atmosphere to prevent evaporation.	

Hill (Hill, 1899) has arranged the methods for preparation of broth, nutrient gelatin, and nutrient agar in tabular form as given on the following page.

For titration, the following method, suggested by Fuller (Fuller, 1895), was adopted by the Public Health Association Committee in 1897 (Committee of Bacteriologists, 1898).

The medium to be tested, all ingredients being dissolved, is brought to the prescribed volume by the addition of distilled water to replace that lost by boiling, and after being thoroughly stirred, 5 c.c. are transferred to a 6-inch porcelain evaporating-dish; to this 45 c.c. of distilled water are added, and the 50 c.c. of fluid are boiled for three minutes over a flame. One c.c. of a .5 per cent solution of phenolphthalein in 50 per cent alcohol is then added and the reaction is determined by titration with $\frac{N}{20}$ sodium

TABLE SHOWING ANALOGY BETWEEN BROTH, NUTRIENT GELATIN, AND NUTRIENT AGAR MADE BY METHODS HEREIN RECOMMENDED.

		Boil 30 gm. thread agar in 1 liter of water for half an hour. Make up to a weight of 1000 gm. *Cool and solidify.*
NUTRIENT BROTH.	NUTRIENT GELATIN.	NUTRIENT AGAR.
1. Infuse lean meat 20 hours with twice its weight of distilled water in refrigerator. Say 1000 gm. meat. " 2000 " water.	Ditto.	Infuse lean meat 20 hours with its own weight of distilled water in refrigerator. Say 1000 gm. meat. " 1000 gm. water.
2. Make up weight of meat infusion (and meat) to original weight by adding water, i.e. to 3000 gm.	Ditto.	Ditto. i.e. to 2000 gm.
3. Filter infusion through cloth to remove meat.	Ditto.	Ditto.
4. Titrate and record reaction of filtrate. Say reaction + 2.2%.	Ditto.	Ditto. Say reaction + 4.2%.
5. Weigh infusion. Say 1800 gm.	Ditto.	Ditto. Say 900 gm.
6. Set infusion on water-bath, keeping temperature below 60° C.	Ditto.	Ditto.
7. Add peptone, 1%, 18 gm.	Ditto and sheet gelatin 10%, 180 gm.	Add peptone, 2%, 18 gm.
8. After ingredients are dissolved, titrate, reaction probably + 2.3 to + 2.5.	Ditto. Probably + 4.0 to + 0.5.	Ditto. Prob. + 4.5 to + 4.7.
9. Neutralize, Fuller's method.	Ditto.	Ditto. To the 900 gm. of meat infusion (containing now peptone and salt) add 900 gm. of the 3% agar jelly described at the head of this column.

10. Heat over boiling water (or steam) bath thirty minutes.
11. Restore weight lost by evaporation to original weight of filtered meat infusion, i.e. that on which the percentage of peptone and salt, etc., were calculated, − 1800 gm. in *each case*.
12. Titrate, reaction probably + 0.3 to + 0.5.
13. Adjust reaction to final point desired, + 1.5 per cent.
14. Boil five minutes over free flame, with stirring.
15. Add water, if necessary, to make up loss by evaporation, to 1800 gm.
16. Filter through absorbent cotton, passing the filtrate through the filter repeatedly until clear.
17. Titrate to determine whether or not the desired reaction has been maintained.
18. Tube and sterilize.

hydroxid or $\frac{N}{20}$ hydrochloric acid. As a rule, the reaction will be acid and the alkali may be used. The determination should be made not less than three times, and the average of the results taken.

LACTOSE AGAR.

This medium is made in the same manner as nutrient agar except that 2 per cent of lactose is added after the final filtration and the reaction is adjusted to $-.5$.

GLYCERINE AGAR.

For this medium, 5 per cent glycerine is added to nutrient agar immediately after final filtration.

SUGAR BROTH.

The most important and widely used sugar broth is that containing dextrose, although lactose and saccharose and occasionally maltose are used for special experimental work.

Dextrose broth may be easily prepared by adding 2 per cent of dextrose to ordinary broth just before the final filtration, and making the reaction neutral. For the preparation of lactose, saccharose, or maltose broth, however, where it is necessary to eliminate dextrose, the meat infusion after filtration is placed in an Erlenmeyer flask, inoculated with an active broth culture of B. coli

and incubated for twenty-four hours at 37° C., whereby the muscle sugar is removed by fermentation.

From this fermented infusion, broth is made in the ordinary way, 2 per cent of the desired sugar added, and the medium tubed and sterilized. All sugar containing media should be sterilized with great care to avoid breaking down the sugar, hence it is advisable to use the discontinuous method, heating to 100° C. for twenty minutes on three successive days, rather than to give a single heating at the very high temperature. There is, however, but little action at 105° C.

It is the custom in some laboratories to prepare broth and sugar solutions separately, and mix the two when a sugar test is to be made.

MILK.

Fresh milk in a tall jar is placed in the refrigerator over night, to allow the cream to rise. The skim-milk is then siphoned off from below the cream, tubed, and sterilized at 110° C. for fifteen minutes, or on three successive days at 100° C. for twenty minutes.

LITMUS MILK.

This is prepared as above, with the addition of ¼ c.c. blue litmus solution to give a faintly alkaline reaction.

If sterilized at a high temperature, reduction of the litmus may take place with loss of color, but oxidation will follow on cooling and exposure to air.

POTATO.

Large, sound potatoes are thoroughly washed and brushed with a scrubbing-brush, then pared and cut into cylinders with a cork-borer. After paring, the potato should be kept under water as much as possible to prevent darkening. The cylinders are then cut diagonally so that each produces two pieces of the general shape of a solidified agar or serum slant. The pieces are then left in cold, running water for several hours, after which they are dropped, broad end down, into test-tubes containing a small piece of glass rod or tubing at the bottom to keep the potato out of the watery fluid which is produced by sterilizing. Sterilize for twenty minutes at 100° C. on three successive days, or for fifteen minutes at 110° C.

NITRATE SOLUTION.

A stock solution is prepared by dissolving 2 grams C. P. potassium nitrate in 100 c.c. sterile distilled water. This should be kept in the ice-chest. Five c.c. of this solution and 1 gram peptone are added to 1 liter of tap-water. The solution is brought to a boil, filtered, and tubed.

Care must be taken that the stock solution does not become reduced to nitrites or that nitrite is not present in the original salt. Sterilize for fifteen minutes at 120°.

PEPTONE SOLUTION.

Ten grams peptone and 5 grams of salt are dissolved in 1 litre of water; the solution is boiled, filtered, tubed, and sterilized for fifteen minutes at 120° C.

LOEFFLER'S BLOOD SERUM.

This medium consists of 3 parts blood serum and 1 part of 1 per cent broth with reaction +0.8. The serum is obtained from fresh beeves' blood, which is collected in sterile jars and allowed to stand for twenty-four hours in the refrigerator for coagulation. The serum is then drawn off, filtered, and mixed with the dextrose broth in the proportion above indicated. Hill finds that filtering the serum through the coagulum obtained after adjusting the reaction of the broth gives a filtrate which is clear and almost colorless (Hill, 1899).

Tubes are filled with the mixture, placed in trays so that the desired slant is obtained, and carefully heated in a Koch coagulator containing cold water in the water-jacket. This water is brought to a boil and kept boiling for three hours. Repeating this process on three successive days solidifies the serum so that it may be subsequently sterilized in flowing steam for twenty minutes on three successive days.

PHENOL BROTH.

To 1000 c.c. water add separately, and in the following order, 100 grams dextrose, 50 grams peptone, 2.5 grams phenol. Heat until all constituents are dissolved, boil for fifteen minutes, and sterilize for fifteen minutes at 110–120° C.

NEUTRAL RED BROTH.

To neutral broth is added 0.5 per cent dextrose and 1 per cent of a 0.5 per cent aqueous solution of Grübler's neutral red. Sterilize at 100°.

MacConkey's Media.

A. Agar.

Agar	1.5 grams
Sodium taurocholate (pure)	0.5 gram
Peptone	2.0 grams
Water	100.0 c.c.

This is boiled, clarified, and filtered as usual, then 1.0 gram lactose is added, and the medium tubed and sterilized for three successive days at 100°.

B. Broth.

Sodium taurocholate (pure)	0.5 gram
Peptone	2.0 grams
Glucose	0.5 gram
Water	100.0 grams

Boil, filter, and add sufficient neutral litmus, fill fermentation-tubes, and sterilize at 100°.

Litmus Solution.—To one-half pound of litmus cubes add enough water to more than cover, boil, and decant off the solution. Repeat this operation with successive small quantities of water until from 3 to 4 liters of water have been used and the cubes are well exhausted of coloring matter. Pour the decantations together and allow them to settle overnight. Siphon off the clear solution. Concentrate to about 1 liter and make the solution decidedly acid with glacial acetic acid. Boil down to about

$\frac{1}{2}$ liter and make exactly neutral with caustic soda or potash. To test for the neutral point, place one drop of the solution in a test-tube. One drop of $\frac{N}{20}$ HCl should turn the drop red, while one drop of $\frac{N}{20}$ NaOH should turn it blue. Filter the solution and sterilize at 110° C. This solution should be added to the media just before use in the proportion of about $\frac{1}{4}$ c.c. to 5 c.c. of medium.

REFERENCES.

ABBA, F. 1895. Sulla presenza del bacillus coli nelle acque potabili, e sopra un metodo per metterlo in evidenza. La Riforma medica, XI, 302.

ABBOTT, A. C. 1899. The Principles of Bacteriology. Philadelphia and New York, 1899.

AMYOT, J. A. 1902. Is the Colon Bacillus a Normal Inhabitant of the Intestines of Fishes? Transactions of the American Public Health Association. 1901 Meeting. XXVII, 400.

ANDREWES, F. W. See LAWS, J. P.

BAKER, S. K. See PRESCOTT, S. C.

BASSETT, V. H. See RUSSELL, H. L.

BECKMANN, W. 1894. Ueber die typhusähnlichen Bakterien des Strassburger Wasserleitungswassers. Archiv für experimentelle Pathologie und Pharmakologie, XXXIII, 466.

BELCHER, D. M. See WINSLOW, C.-E. A.

BELITZER. 1899. Zur Lehre über das Bacterium coli commune. Rev. Jahresbericht über die Fortschritte in der Lehre von den pathogenen Mikroörganismen, XV, 326.

BLACHSTEIN. 1893. Contribution à l'étude microbique de l'eau Annales de l'Institut Pasteur, VII, 689.

BLUNT, T. P. See DOWNES, A.

BOLTON, M. 1886. Ueber das Verhalten verschiedener Bakterienarten im Trinkwasser. Zeitschrift für Hygiene, I, 76.

BROTZU, L. 1895. Sulla desinfezione del canale intestinale. Annali dell' istituto d'igiene sperimentale della R. Università di

Roma, IV, 427. Rev. Centralblatt für Bakteriologie, XVII, 726.

BROWN, C. C. 1893. Fourth Report on Mohawk and Hudson Rivers. Thirteenth Annual Report of the State Board of Health of New York for 1892, 680.

BRUNS, H. See LEVY, E.

BUCHNER. 1893. Ueber den Einfluss des Lichtes auf Bakterien und über die Selbstreinigung der Flüsse. Achiv für Hygiene, XVII, 179.

BURRI, R. 1895. Nachweis von Fäkalbakterien im Trinkwasser. Hygienische Rundschau, V, 49.

CAMBIER, R. See MIQUEL, P.

CHICK, H. 1901. The Distribution of Bacterium coli commune. The Thompson-Yates Laboratories Report, III, 1 and 117.

CLARK, H. W., and GAGE, S. DEM. 1900. The Significance of the Appearance of B. coli communis in Filtered Water. Journal of the Boston Society of Medical Sciences, IV, 172.

COMMITTEE OF BACTERIOLOGISTS. 1898. Procedures recommended for the Study of Bacteria, with especial Reference to Greater Uniformity in the Description and Differentiation of Species. Being the Report of a Committee of Bacteriologists to the Committee on the Pollution of Water-supplies of the American Public Health Association. Transactions of the American Public Health Association. 1897 Meeting. XXIII, 60.

COPELAND, W. R. 1901. The Use of Carbolic Acid in Isolating the Bacillus coli communis from River Water. Journal of the Boston Society of Medical Sciences, V, 381.

CRAMER, C. 1885. Die Wasserversorgung von Zürich und ihr Zusammenhang mit den Typhus-epidemie des Jahres 1884. Zurich.

DOMBROWSKY. 1903. Zur Biologie der Ruhrbacillen. Archiv für Hygiene, XLVII, 243.

DOWNES, A., and BLUNT, T. P. 1877. Researches on the Effect of Light upon Bacteria and other Organisms. Proceedings of the Royal Society, XXVI, 488.

DUCLAUX, E. 1898. Traité de Microbiologie, Vol. I. Paris, 1898.

DUNBAR. 1896. Zur Differentialdiagnose zwischen den Cholera-vib ionen und anderen denselben nahestehenden Vibrionen. Zeitschrift für Hygiene, XXI, 295.

DUNHAM, E. K. 1887. Zur chemischen Reaction der Cholera-bakterien. Zeitschrift für Hygiene, II, 337.

DUNHAM, E. K. 1889. On the Bacteriological Test of Drinking-water. Medical Record, XXXVI, 367.

DYAR, H. G., and KEITH, S. C. 1893. Notes on Normal Intestinal Bacilli of the Horse and of certain other Domesticated Animals. Technology Quarterly, VI, 256.

ELLMS, J. W. See HILL, H. W.

ELSNER. 1896. Untersuchungen über electives Wachsthum der Bacterium coli-arten und des Typhusbacillus. Zeitschrift für Hygiene, XXI, 25.

ESCHERICH, T. 1885. Die Darmbakterien des Neugeborenen und Säuglings. Fortschritte der Medicin, III, 515 and 547.

FISCHER, B., and FLATAU, G. 1901. Typhusbacillen in einer eingesandten typhusverdächtigen Wasserprobe. Centralblatt für Bakteriologie, XXIX, 329.

FLATAU, G. See FISCHER, B.

FRANKLAND, G. and P. 1894. Micro-organisms in Water. London, 1894.

FREMLIN. 1893. Vergleichende Studien an Bact. coli commune verschiedener Provenienz. Archiv für Hygiene, XIX, 295.

FREUDENREICH, E. VON. 1888. De l'antagonisme des bactéries et de l'immunité qu'il confère aux milieux de culture. Annales de l'Institut Pasteur, II, 200.

FREUDENREICH, E. VON. 1895. Ueber den Nachweis des Bacillus coli communis im Wasser und dessen Bedeutung. Centralblatt für Bakteriologie, XVIII, 102.

FROST, W. D. 1901. A Laboratory Guide in Elementary Bacteriology. Madison, 1901.

FULLER, G. W. 1895. On the Proper Reaction of Nutrient Media for Bacterial Cultivation. Transactions of the American Public Health Association. 1894 Meeting. XX, 381.

FULLER, G. W. 1899. Report on the Investigations into the Purification of the Ohio River Water for the Improved

Water-supply of the City of Cincinnati, Ohio. Cincinnati, 1899.

FULLER, G. W. 1902. Report of Committee on Standard Methods of Water Analysis. Transactions of the American Public Health Association. 1901 Meeting. XXVII, 377.

FULLER, G. W., and JOHNSON, G. A. 1899. On the Differentiation and Classification of Water Bacteria. Journal of Experimenta-Medicine, IV, 609.

GAGE, S. DEM. 1901. Notes on Testing for B. coli in Water. Journal of Applied Microscopy, IV, 1403.

GAGE, S. DEM. 1902. Bacteriological Studies at the Lawrence Experiment Station, with Special Reference to the Determination of B. coli. Thirty-third Annual Report of the State Board of Health of Massachusetts for 1901, 397.

GAGE, S. DEM., and PHELPS, E. B. 1902. Studies of Media for the Quantitative Estimation of Bacteria in Water and Sewage. Transactions of the American Public Health Association. 1901 Meeting. XXVII, 392.

GAGE, S. DEM., and PHELPS, E. B. 1903. Notes on B. coli and Allied Forms, with Special Reference to the Neutral Red Reaction. Transactions of the American Public Health Association. 1902 Meeting. XXVIII, 402.

GAGE, S. DEM. See CLARK, H. W.

GARRÉ, C. 1887. Ueber Antagonisten unter den Bakterien. Correspondenzblatt für Schweizer Aertze. XVII, 385.

GÄRTNER, A. See TIEMANN, F.

GOTSCHLICH, E. See KOLLE, W.

HAMMERL, H. 1897. Ueber das Vorkommen des Bacterium coli im Flusswasser. Hygienische Rundschau, VII, 529.

HANKIN, E. H. 1899. On the Detection of the B. typhi abdominales in Water and other Substances. Centralblatt für Bakteriologie, XXVI, 554.

HANSEN, P. See WOODMAN, A. G.

HEIDER, A. 1893. Untersuchungen über die Verunreinigungen der Donau durch die Abwässer der Stadt Wien. Das österreichische Sanitätswesen. V, 53.

HERAEUS, W. 1896. Ueber das Verhalten der Bakterien im

Brunnenwasser, sowie über reducirende und oxydirende Eigenschaften der Bakterien. Zeitschrift für Hygiene, I, 193.

HESSE, W., and NIEDNER. 1898. Die Methodik der bakteriologischen Wasseruntersuchung. Zeitschrift für Hygiene, XXIX, 454.

HILL, C. A. See MACCONKEY, A.

HILL, H. W., and ELLMS, J. W. 1898. An Apparatus for the Collection of Samples of Water for Chemical, Microscopical, and Bacteriological Analysis. Transactions of the American Public Health Association. 1897 Meeting. XXIII, 193.

HILL, H. W. 1899. Report of Director of Bacteriological Laboratory. Twenty-seventh Annual Report of the Health Department of the City of Boston for the year 1898, 63.

HORROCKS, W. H. 1901. An Introduction to the Bacteriological Examination of Water. London, 1901.

HOUSTON, A. C. 1898. Chemical and Bacteriological Examination of Soils with reference to the Amount and Nature of the Organic Matter and the Number and Character of the Bacteria contained in them. Supplement to the Twenty-seventh Annual Report of the Local Government Board, containing the Report of the Medical Officer for 1897–98, 251.

HOUSTON, A. C. 1899. *a*. Chemical and Bacteriological Examination of the "Washings" of Soils, with Reference to the Amount and Nature of the Organic Matter and the Number and Character of the Bacteria contained in them. Supplement to the Twenty-eighth Annual Report of the Local Government Board containing the Report of the Medical Officer for 1898–9, 439.

HOUSTON, A. C. 1899. *b*. Bacterioscopic Examination of Drinking-water with Particular Reference to the Relations of Streptococci and Staphylococci with Water of this Class. Supplement to the Twenty-eighth Annual Report of the Local Government Board containing the Report of the Medical Officer for 1898–9, 467.

HOUSTON, A. C. 1900. *a*. Chemical and Bacteriological Examination of the "Washings" of Soils, with Reference to the Amount and Nature of the Organic Matter, and the Number

and Character of the Bacteria contained in them. Supplement to the Twenty-ninth Annual Report of the Local Government Board containing the Report of the Medical Officer for 1899–1900, 489.

HOUSTON, A. C. 1900. *b*. On the Value of Examination of Water for Streptococci and Staphylococci with a view to detection of its Recent Contamination with Animal Organic Matter. Supplement to the Twenty-ninth Annual Report of the Local Government Board containing the Report of the Medical Officer for 1899–1900, 458.

HOUSTON, A. C. 1901. Report on the Chemical and Bacteriological E amination of Chichester Well-water. Supplement to the Thirtieth Annual Report of the Local Government Board containing th Report of the Medical Officer for 1900–01, 511.

HOUSTON, A. C. See KLEIN, E.

HUNNEWELL, M. P. See WINSLOW, C.-E. A.

IRONS, E. E. 1901. Some Observations on Methods for the Detection of B. coli communis in Water. Transactions of the American Public Health Association, 1900 Meeting, XXVI, 310.

IRONS, E. E. 1902. Neutral Red in the Routine Examination of Water. Journal of Hygiene, II, 314.

JANOWSKI, T. 1888. Ueber den Bakteriengehalt des Schnees. Centralblatt für Bakteriologie. IV, 547.

JOHNSON, G. A. See FULLER, G. W.

JORDAN, E. O. 1890. A Report on Certain Species of Bacteria observed in Sewage. Special Report of the State Board of Health of Massachusetts on the Purification of Sewage and Water, II, 821.

JORDAN, E. O. 1900. Some Observations upon the Bacterial Self-purification of Streams. Journal of Experimental Medicine. V, 271.

JORDAN, E. O. 1901. The Relative Abundance of Bacillus coli communis as an Index of the Self-purification of Streams. Journal of Hygiene, I, 295.

KEITH, S. C. See DYAR, H. G.

KLEIN, E. 1898. Report on the Morphology and Biology of

Bacillus enteritidis sporogenes; on the association of this microbe with Infantile Diarrhœa and with Cholera Nostras; and on its relations with Milk, with Sewage, and with Manure. Supplement to the Twenty-seventh Annual Report of the Local Government Board containing the Report of the Medical Officer for 1897–8, 210.

KLEIN, E. 1899. Further Report on the Bacillus enteritidis sporogenes. Supplement to the Twenty-eighth Annual Report of the Local Government Board containing the Report of the Medical Officer for 1898–9, 312.

KLEIN, E., and HOUSTON, A. C. 1898. Report on Bacteriological Evidence of Presumably Recent and therefore Dangerous Sewage Pollution of elsewise Potable Waters. Supplement to the Twenty-seventh Annual Report of the Local Government Board containing the Report of the Medical Officer for 1897–8, 318.

KLEIN, E., and HOUSTON, A. C. 1899. Further Report on Bacteriological Evidence of Recent and therefore Dangerous Sewage Pollution of elsewise Potable Waters. Supplement to the Twenty-eighth Annual Report of the Local Government Board containing the Report of the Medical Officer for 1898–9, 498.

KLEIN, E., and HOUSTON, A. C. 1900. Preliminary Account of the Results of a Bacterioscopic Analysis of Different Cereals and Foodstuffs. Supplement to the Twenty-ninth Annual Report of the Local Government Board containing the Report of the Medical Officer for 1899–1900, 593.

KOCH, R. 1893. Ueber den augenblicklichen Stand der bakteriologischen Choleradiagnose. Zeitschrift für Hygiene, XIV, 319.

KOLLE, W., and GOTSCHLICH, E. 1903. Untersuchungen über die Bakteriologische Choleradiagnostik und Specificität der Kochschen Choleravibrio. Zeitschrift für Hygiene, XLIV, 1.

KRUSE, W. 1894. Kritische und experimentelle Beiträge zur hygienischen Beurtheilung des Wassers. Zeitschrift für Hygiene XVII, 1.

KÜBLER and NEUFELD, F. 1899. Ueber einen Befund von Typhusbacillen im Brunnenwasser. Zeitschrift für Hygiene, XXXI, 133.

KÜSEL, G. C. See PENNINGTON, M. E.

LAWS, J. P., and ANDREWES, F. W. 1894. Report on the Result of Investigations of the Micro-organisms of Sewage. Report to London County Council, December 13, 1894.

LE GROS, F. L. 1902. Monographie des Streptocoques et des agents des septicémies métadiphtériques particulièrement des diplocoques. Paris, 1902.

LEVY, E., and BRUNS, H. 1899. Zur Hygiene des Wassers. Archiv für Hygiene, XXXVI, 178.

LOEFFLER, 1893. Zum Nachweis der Cholerabakterien im Wasser. Centralblatt für Bakteriologie, XIII, 380.

MACCONKEY, A. 1900. Experiments on the Differentiation and Isolation from Mixtures of the Bacillus coli communis and Bacillus typhosus by the Use of Sugars and the Salts of Bile. The Thompson-Yates Laboratories Report, III, 41.

MACCONKEY, A. 1901. Further Note on Bile Salt Lactose Agar. The Thompson-Yates Laboratories Report, III, 151.

MACCONKEY, A., and HILL, C. A. 1901. A Simple Test for Fæcal Contamination. The Thompson-Yates Laboratories Report, IV, 151.

MAKGILL, R. H. 1901. The Neutral-red Reaction as a means of detecting Bacillus coli in Water-supplies. Journal of Hygiene, I, 430.

MASCHEK, J. 1887. Bakteriologische Untersuchungen des Leitmeritzer Trinkwasser. Jahresbericht der Oberrealschule zu Leitmeritz. 1887.

MASSACHUSETTS STATE BOARD OF HEALTH, 1899. Thirtieth Annual Report, for 1898.

MASSACHUSETTS STATE BOARD OF HEALTH, 1900. Thirty-first Annual Report, for 1899.

MASSACHUSETTS STATE BOARD OF HEALTH, 1901. Thirty-second Annual Report, for 1900.

MASSACHUSETTS STATE BOARD OF HEALTH, 1902. Thirty-third Annual Report, for 1901.

MATHEWS, A. P. 1893. On Wurtz's Method for the Differentiation of Bacillus typhi abdominalis from Bacillus coli communis and its Application to the Examination of Contaminated Drinking-water. Technology Quarterly, VI, 241.

MIQUEL, P. 1886. De la richesse en bactéries des eaux d'essangeage. Revue d'Hygiene, VIII, 388.

MIQUEL, P. 1891. Manuel pratique d'analyse bactériologique des eaux. Paris, 1891.

MIQUEL, P., and CAMBIER, R. 1902. Traité de Bactériologie pure et appliquée. Paris, 1902.

MOORE, V. A., and WRIGHT, F. R. 1900. A Comparison of B. coli communis from Different Species of Animals. Journal of the Boston Society of Medical Sciences, IV, 175.

MOORE, V. A., and WRIGHT, F. R. 1902. Preliminary Observations on B. coli communis from Certain Species of Animals. Science, N. S., XV, 372.

MORONI, A. 1898. Bacterium coli commune. Archives per le Scienze mediche, XXII, 261.

MORONI, A. 1899. La presenza del bacillus coli communis nelle acque. La Riforma medica, XV, 111.

NEUFELD, F. See KÜBLER.

NIBECKER, C. P. See WINSLOW, C.-E. A.

NIEDNER. See HESSE, W.

NUTTALL, G. H. F. See WELCH, W. H.

PAKES, E. C. C. 1900. The Application of Bacteriology to Public Health. Public Health, XII, 385.

PAPASOTIRIU, J. 1901. Untersuchungen über das Vorkommen des Bact. coli in Teig, Mehl und Getreide, nebst einigen Bemerkungen über die Bedeutung des Bact. coli als Indikator für Verunreinigung von Wasser mit Fäkalein. Archiv. für Hygiene, XLI, 204.

PARIETTI, E. 1890. Metodo di ricerca del Bacillo del tifo nelle acque potabili. Rivista d'Igiene e Sanita publica. I, 409.

PENNINGTON, M. E., and KÜSEL, G. C. 1900. An Experimental Study of the Gas-producing Power of Bacillus coli communis under Different Conditions of Environment. Journal of the American Chemical Society, XXII, 556.

PETRUSCHKY, J., and PUSCH, H. 1903. Bacterium coli als Indicator für Fäkalverunreinigung von Wässern. Zeitschrift für Hygiene, XLIII, 304.

PHELPS, E. B. See GAGE, S. D.

POUJOL, M. G. 1897. Sur la présence très fréquente du Bacterium coli dans les eaux naturelles. Comptes rendus de la Société de Biologie, IV, 982.

PRAUSNITZ, W. 1890. Der Einfluss der Münchener Kanalisation auf die Isar mit besonderer Berücksichtigung der Frage der Selbstreinigung der Flüsse. Munich, 1890.

PRESCOTT, S. C. 1902. *a*. On the Apparent Identity of the Cultural Reactions of B. coli communis and certain Lactic Bacteria. Science, N. S., XV, 363.

PRESCOTT, S. C. 1902. *b*. A Note on Methods of Isolating Colon Bacilli. Science, N. S., XVI, 671.

PRESCOTT, S. C. 1903. On Certain Precautions Required in Making and Interpreting the so-called "Colon Test" for Potable Waters. Medicine, XI, 20.

PRESCOTT, S. C., and BAKER, S. K. 1904. The Cultural Relations of Bacillus coli and Houston's Sewage Streptococci, and a Method for the Detection of these Organisms in Polluted Waters. Journal of Infectious Diseases, I, 193.

PRESCOTT, S. C. See SEDGWICK, W. T.

PROCACCINI, R. 1893. Influenza della luce solare sulle acque di rifiuto. Annali dell' Istituto d'Igiene Sperimentale di Roma, III, 437.

PUSCH, H. See PETRUSCHKY, J.

RAPP, R. 1903. Ueber den Einfluss des Lichtes auf organische Substanzen mit besonderer Berücksichtigung der Selbstreinigung der Flüsse. Archiv. für Hygiene, XLVIII, 179.

REFIK. 1896. Sur les divers types de coli-bacille des eaux. Annales de l'Institut Pasteur, X, 242.

REINSCH, A. 1891. Zur bakteriologischen Untersuchung des Trinkwassers. Centralblatt für Bakteriologie, X, 415.

REMLINGER, P., and SCHNEIDER, G. 1897. Contribution à l'Étude du bacille typhique. Annales de l'Institut Pasteur, XI, 55.

REYNOLDS, A. R. 1902. Report of Streams Examination, Chemic and Bacteriologic, of the Waters between Lake Michigan at Chicago and the Mississippi River at St. Louis, for the purpose of determining their condition and quality before and after the Opening of the Drainage Channel. Chicago, 1902.

RIDEAL, S. 1902. Water and its Purification. London, 1902.

RIEDEL. See WOLFFHÜGEL.

ROTHBERGER, C. J. 1898. Differential diagnostische Untersuchungen mit gefärbten Nährböden. Centralblatt für Bakteriologie, Abth. I, XXIV, 513.

RUSSELL, H. L. 1891. Untersuchungen über im Golf von Neapel lebende Bakterien. Zeitschrift für Hygiene, XI, 166.

RUSSELL, H. L. 1892. Bacterial Investigation of the Sea and its Floor. Botanical Gazette, XVII, 312.

RUSSELL, H. L., and BASSETT, V. H. 1899. The Significance of Certain Gas-forming Bacteria of Non-colon Type in Sanitary Water Analysis. Proceedings of American Public Health Association, XXV, 1899 Meeting, 570.

SAVAGE, W. G. 1901. Neutral Red in the Routine Bacteriological Examination of Water. Journal of Hyigene, I, 437.

SAVAGE, W. G. 1903. The Pathogenicity of B. coli in Relation to the Bacteriological Examination of Water. Journal of Hygiene, III, 388.

SCHEURLEN. 1891. Ueber die Wirkung des Centrifugirens auf Bakteriensuspensionen, besonders auf die Vertheilung der Bakterien in der Milch. Arbeiten aus dem Kaiserlichen Gesundheitsamte, VII, 269.

SCHNEIDER. See REMLINGER.

SCHOTTELIUS, M. 1885. Zum mikroskopischen Nachweis von Cholerabacillen in Dejectionen. Deutsche medicinische Wochenschrift, XI, 213.

SEDGWICK, W. T. 1889. Recent Progress in Biological Water Analysis. Journal of the New England Water-works Association, IV, 1.

SEDGWICK, W. T. 1902. Principles of Sanitary Science. New York, 1902.

SEDGWICK, W. T., and PRESCOTT, S. C. 1895. *a*. On the Bacterial Contents of Certain Ground-waters, including Deep Wells. Twenty-sixth Annual Report of the State Board of Health of Massachusetts for 1894, 435.

SEDGWICK, W. T., and PRESCOTT, S. C. 1895. *b*. On the Influence of Slight Variations in the Composition of Nutrient Gelatin

upon the Development of Water Bacteria. Transactions of the American Public Health Association, 1894 Meeting, XX, 450.

SEDWICK, W. T., and WINSLOW, C.-E. A. 1902. Experiments on the Effect of Freezing and other Low Temperatures upon the Viability of the Bacillus of Typhoid Fever, with Considerations regarding Ice as a Vehicle of Infectious Disease. Memoirs of the American Academy of Arts and Sciences, XII, 467.

SHUTTLEWORTH, E. B. 1895. Some Deductions from Bacteriological Work on the Water of Lake Ontario. Transactions of the American Public Health Association, 1894 Meeting, XX, 58.

SMITH, T. 1893. *a*. A New Method for Determining Quantitatively the Pollution of Water by Fæcal Bacteria. Thirteenth Annual Report of the State Board of Health of New York for 1892, 712.

SMITH, T. 1893. *b*. The Fermentation-tube with Special Reference to Anaerobiosis and Gas Production among Bacteria. The Wilder Quarter-century Book, 187.

SMITH, T. 1895. Ueber den Nachweis des Bacillus coli communis im Wasser. Centralblatt für Bakteriologie, XVIII, 494.

STERNBERG, G. M. 1892. Manual of Bacteriology. New York, 1892.

THOMSON, T. 1894. Report on an Epidemic of Enteric Fever in the Borough of Worthing and in the Villages of Broadwater and West Tarring. Supplement to the Twenty-third Annual Report of the Local Government Board, containing the Report of the Medical Officer for 1893–4, 47.

TIEMANN, F., and GÄRTNER, A. 1889. Die chemische und mikroscopisch-bakteriologische Untersuchung des Wassers. Brunswick, 1889.

WATHETET, M. A. 1895. Recherches bactériologiques sur les déjections dans la fièvre typhoïde. Annales de l'Institut Pasteur, IX, 251.

WEISSENFELD, J. 1900. Der Befund des Bacterium coli im Wasser und das Thierexperiment sind keine brauchbare Hülfsmittel für die hygienische Beurtheilung des Wassers. Zeitschrift für Hygiene, XXXV, 78.

WELCH, W. H., and NUTTALL, G. H. F. 1892. A Gas-producing Bacillus (Bacillus aerogenes capsulatus, N. S.) Capable of Rapid Development in the Blood Vessels after Death. Bulletin of the Johns Hopkins Hospital, July, 1892.

WHIPPLE, G. C. 1896. Discussion of Paper on Water-borne Diseases, by T. Smith. Journal of the New England Water-works Association, X, 217.

WHIPPLE, G. C. 1899. On the Necessity of Cultivating Water Bacteria in an Atmosphere Saturated with Moisture. Technology Quarterly, XII, 276.

WHIPPLE, G. C. 1901. Changes that take place in the Bacterial Contents of Water during Transportation. Technology Quarterly, XIV, 21.

WHIPPLE, G. C. 1902. On the Physical Properties of Gelatin with Reference to its Use in Culture Media. Technology Quarterly, XV, 127.

WHIPPLE, G. C. 1903. On the Practical Value of Presumptive Tests for Bacillus coli in Water. Technology Quarterly, XVI, 18.

WIDAL, F. 1896. Sérodiagnostic de la fièvre typhoïde. La Semaine Médicale, XVI, 259.

WINSLOW, C.-E. A. 1901. The Bacteriological Examination of Water and its Interpretation. Journal of the New England Water-works Association, XV, 459.

WINSLOW, C.-E. A. 1903. *a*. Studies on Quantitative Variations in Gas Production in the Fermentation-tube. Science, N. S., XVII, 375.

WINSLOW, C.-E. A. 1903. *b*. The Occurrence of the Colon Bacillus on the Hands. Journal of Medical Research, X, 463.

WINSLOW, C.-E. A., and BELCHER, D. M. 1904 Changes in the Bacterial Flora of Sewage during Storage. Journal of Infectious Diseases, I, 170.

WINSLOW, C.-E. A., and HUNNEWELL, M. P. 1902. *a*. Streptococci Characteristic of Sewage and Sewage-polluted Waters. Science, N. S., XV, 827.

WINSLOW, C.-E. A., and HUNNEWELL, M. P. 1902. *b*. A Study of the Distribution of the Colon Bacillus of Escherich and of the

Sewage Streptococci of Houston in Polluted and Unpolluted Waters. Journal of Medical Research, VIII, 502.

WINSLOW, C.-E. A., and NIBECKER, C. P. 1903. The Significance of Bacteriological Methods in Sanitary Water Analysis. Technology Quarterly, XVI, 227.

WINSLOW, C.-E. A. See SEDWICK, W. T.

WINSLOW, C.-E. A. See WOODMAN, A. G.

WOLFFHÜGEL, G. 1886. Erfahrungen über den Keimgehalt brauchbarer Trink- und Nutzwasser. Arbeiten aus dem Kaiserlichen Gesundheitsamte, I, 546.

WOLFFHÜGEL, G., and RIEDEL, O. 1886. Die Vermehrung der Bakterien im Wasser. Arbeiten aus dem Kaiserlichen Gesundheitsamte, I, 455.

WOODMAN, A. G., WINSLOW, C.-E. A., and HANSEN, P. 1902. A Study of Self-purification in the Sudbury River. Technology Quarterly, XV, 105.

WRIGHT, F. R. See MOORE, V. A.

WURTZ, R. 1892. Note sur deux caractères différentiels entre le bacille d'Eberth et le B. coli commune. Archives de Médecine Expérimentale, IV, 85.

ZAGARI, G. 1887. Esperienze sulla concorrenza vitale dei microorganismi e sopra un nuovo mezzo di profilassi carbonchiosa. Giornale internazionale della Scienze Mediche, IX, 617.

AUTHOR INDEX.

PAGE

Abba 78
Abbott 25
Amyot 75
Andrewes 51, 54, 100

Baker 102
Bassett 75
Beckmann 76
Belcher 107
Belitzer 75
Blachstein 78
Blunt 13
Bolton 37
Brotzu 75
Brown 83
Bruns 78
Buchner 13
Burri 82

Cambier 25, 31
Chick 45, 60, 85
Clark 40, 87
Copeland 60
Cramer 38

Dombrowsky 53
Downes 13
Duclaux 7
Dunbar 55

PAGE
Dunham 40, 55
Dyar 74

Egger 15
Ellms 25
Elsner 50
Escherich 58, 74

Fischer 15, 53
Flatau 53
Frankland 9, 10, 25, 54, 56
Fremlin 74
Freudenreich 12, 82
Frost 70
Fuller 20, 29, 40, 73, 85, 119, 120

Gage 19, 21, 30, 61, 63, 66, 72, 73, 87, 95, 98, 101
Garré 12
Gärtner 23, 37
Gotschlich 56

Hammerl 83
Hankin 50
Hansen 113
Heider 116, 117
Heræus 23
Hesse 19
Hill 25, 98, 120, 125
Hoag 75
Horrocks 12, 15, 85, 101, 105
Houston 80, 84, 100, 105, 107, 113, 115
Hunnewell 64, 91, 96, 101

Irons 61, 63, 95, 98

Janowski 8
Johnson 73
Jordan 11, 13, 14, 27, 39, 88, 89, 117

Keith 74
Klein 50, 80, 106, 107, 113
Koch 55

PAGE
Kolle 56
Kruse 76
Kübler 53
Küsel 73

Laws 51, 54, 100
Le Gros 101
Levy 78
Loeffler 55, 125

MacConkey 98, 126
Makgill 97
Maschek 15, 23
Mathews 46, 59
Miquel 8, 9, 25, 31, 35, 39, 82
Moore 75
Moroni 78

Neufeld 53
Nibecker 10, 46, 66, 97, 101
Niedner 19
Nuttall 106

Osgood 116

Pakes 84
Papasotiriu 80
Parietti 49
Pennington 73
Petruschky 92
Phelps 19, 21, 30, 66, 72, 98
Poujol 77
Prausnitz 39
Prescott 16, 17, 29, 61, 80, 101, 102
Procaccini 13
Pusch 92

Rapp 13, 14
Refik 76
Reinsch 29
Remlinger 54

PAGE
Reynolds..... 62
Rideal..... 46
Riedel..... 26
Rothberger..... 97
Russell..... 75

Savage..... 97
Scheurlen..... 11
Schneider..... 54
Schottelius..... 55
Sedgwick.....5, 12, 16, 17, 29, 57, 59
Shuttleworth..... 41
Smith.....60, 74, 82, 94
Sternberg..... 36
Stokes..... 98

Thomson..... 50
Tiemann.....23, 37
Tissandier..... 7

Wathelet..... 51
Weissenfeld..... 78
Welch..... 106
Whipple.....9, 20, 26, 30, 31, 58, 65, 68, 96, 113, 115
Widal..... 53
Winslow.....10, 12, 22, 41, 46, 64, 66, 73, 91, 96, 97, 101, 107, 113
Wollfhügel.....15, 26
Woodman..... 113
Wright..... 75
Wurtz.....44, 59

Zagari..... 12

SUBJECT INDEX.

PAGE

Absorption of CO_2 70
Acid formers in Boston sewage..... 45
    production by B. subtilis..... 45
Acidity of media..... 29
Agar media..... 30
    plate count..... 44
    preparation of..... 120, 121
    streak, types of growth..... 67
Agglutination..... 53
Air, dust in 7
American Public Health Association, standard procedure... 19, 20, 28, 30, 31, 119, 120
Amines, formation of by B. coli..... 66
Ammonia, formation of by B. coli..... 66
Ammonium compounds, decomposition of..... 5
Antagonism..... 12

Bacillus ærogenes 108
    anthracis, isolation from water..... 56
    cloacæ..... 91, 92, 108
    coli, as a saprophyte..... 76
        , as direct evidence of sewage 58
        , atypical forms..... 68
        , biochemical characters..... 51, 58, 59
        , characteristics of..... 67
        , colonies on agar..... 67
        , comparison of methods for isolation..... 61, 63
        , decomposition of sugars..... 44
        , detection of, in presence of streptococci..... 104

PAGE
Bacillus coli, determined by animal inoculation..... 78
, distribution of..... 81
, distribution of, in unpolluted waters..... 76–79
, distribution of, in various animals..... 74
, distribution in various waters..... 85
, effect of dilution on growth in litmus lactose agar.... 66
, from cereals..... 82
, gas ratio..... 95
, general distribution of, in nature..... 76
, growth on agar streak..... 67
in effluents of Lawrence filter..... 87
in filter effluents..... 86
in large samples..... 82
in Merrimac River..... 86
in mud..... 45
in normal intestine..... 58
in polluted waters..... 89, 91
in refuse from tanneries, grist-mills, and dairies..... 82
in river water..... 83, 92
in Severn River..... 45
in sewage..... 89, 92
in soil..... 84
in spring water..... 78, 88
in stored sewage..... 101
, in unpolluted waters..... 78, 91, 97
in well waters 78, 92
, isolation of, by Escherich..... 58
, isolation of pure cultures..... 65
, isolation of, by litmus lactose agar..... 59
, morphology of..... 58
, on cereals..... 80
, on hands..... 22
, overgrowth by streptococci..... 102, 103, 104
, overgrowth during preliminary incubation 62, 64, 65
, pathogenesis for guinea-pigs..... 59
, pathogenicity of..... 78
, percentage of cultures giving positive tests in sub-culture..... 72
, positive isolations by different methods..... 63
, presumptive tests for..... 94

PAGE
Bacillus coli, presumptive tests in various amounts of water..... 96
, qualitative analysis for..... 69, 70
, quantitative estimation of 35, 82
, relation to B. acidi lactici..... 80
, significance of..... 112
, significance of, in samples of different size..... 87
, significance of, in water. 74
, standards for drinking water 85
, standards for presumptive test..... 97
, sub-cultures of, in qualitative analysis..... 70
, susceptibility to phenol 59
, time of gas-production in dextrose broth by..... 61
, variations in cultural reactions..... 73
, varieties of..... 68
Bacillus dysenteriæ..... 53
enteritidis sporogenes..... 106
mycoides..... 66
, growth on agar streak..... 67
sporogenes, as index of pollution..... 106
, cultural characteristics..... 106, 107
in sewage..... 107
, method for detection of, in water..... 106
, significance of..... 107
typhi, biochemical characters of..... 51
, distribution in the environment..... 54
in ice..... 12
, isolation of..... 49
, life of, in water..... 54
Bacteria, classes of, in relation to nutrition..... 37
, comparative numbers of, growing at 20° and 37°..... 47
, definition of species..... 67
, developing on various media..... 30
, distribution of 3
, distribution of, in sea-water..... 10
, distribution in water..... 6
, diurnal variation..... 14
fermenting lactose, in normal waters..... 46, 48
, in polluted waters..... 45, 48
growing at body temperature..... 43
in Boston tap-water..... 9, 38

PAGE

Bacteria in Boston sewage 45
in Cambridge supply 10
in Chicago drainage canal 39
in Connecticut River 42
in deep wells 17, 40
in driven wells 17, 40
in filter effluents 40
in Framingham supply 42
in ground water 15, 39
in ground waters, peculiar character of 18, 39
in Hartford supply 42
in ice 12
in Isar River 39
in Lake Champlain 10
in Lake of Lucerne 10
in Lake Zurich 38
in lakes and ponds 10
in Loch Katrine 10
in Loch of Lintralthen 10
in Lynn supply 10
in Medford supply 10
in Merrimac River 9
in Newport supply 42
in Ohio River 41
in Ourcq River 9
in Peabody supply 10
in Plymouth supply 10
in polluted streams 39
in polluted waters, changes during storage 27
in polluted well 42
in rain 8
in relation to food supply 14
in Salem supply 10
in sea-water at Naples 10
in sea-water at Wood's Hole 10
in Seine 39
in shallow wells 15, 16
in snow 8
in springs 15, 16
in stored samples, multiplication of 25–27

PAGE
Bacteria in surface wash 8
in surface waters 9
in Taunton supply 10
in Thames River 9
in Wakefield supply 10
in water, relation to organic matter 37, 38, 111
, significance of number of 38
in well water, effect of pumping on 23
, intestinal 99
, liquefying 108
, metabolism of 4
, multiplication of, in spring water 16
, peculiar groups in ground water 18, 39
, reduction of, in streams 8
, relation to environment 3, 14
organic matter in water 6
waste materials 4
, seasonal distribution in surface waters 9
which do not grow on ordinary media 19
Bacterial standards for filter plants 41
Bacteriological analyses, value of 41
analysis of water, certainty of 42, 115
, comparison with chemical 113
, delicacy of 113
, directness of 113
, distribution of sewage in water 116
ground waters 113, 114, 115
examination of water, applicability of 116
Berkefeld filter to concentrate organisms 50
Biochemical characters of B. coli 51
Blood serum, preparation of 125
Body temperature, count, Boston sewage 45
, Charles River 45
of various samples 47
, polluted water 45
, significance of 112
organisms growing at 43
Boston, B. sporógenes in sewage 107
, bacteria in deep wells 17
, course of sewage in harbor 116

PAGE
Boston sewage, bacteria in..... 45
tap-water, bacteria in..... .....9, 38
Brookline tap-water, bacteria in..... 47
Brooks, bacteria in... 47

Cambridge tap-water, bacteria in..... 10, 47
Carbon dioxide, absorption of..... 70
Cats, B. coli in.....74, 75
Cereals, B. coli in..... 80
Character of colonies, importance of, in ground waters..... 40
Charles River above Boston, body temperature, count..... 45
Chemical analysis of water, comparison with bacteriological..... 113
Chemical examination of water..... 110
, applicability of..... 116
Chicago drainage canal, B. coli in..... 89
, bacteria in 39
, routine bacteriological analyses..... 41
Chichester, well waters in..... 115
Cleaning mixtures..... 22
Cold, action of, upon bacteria..... 12
Colon bacilli in Manchester Ship Canal..... 45
, overgrown by streptococci..... 92
Colon bacillus, as direct evidence of sewage..... 58
, biochemical characters.....58, 59
, gas ratio..... 58
, importance of number of..... 82
, in normal intestine..... 58
, isolation of, by litmus lactose agar..... 59
, morphology of 58
, pathogenesis in guinea-pigs 59
, preliminary enrichment for..... 60
, quantitative estimation of..... 60
, susceptibility to phenol..... 59
Comparability of quantitative results..... 20
Composition of media, effect on bacterial counts..... 30
Connecticut River, bacteria in..... 42
Constantinople, B. coli in water supplies 76
Counting.....32, 33
Cows, B. coli in.....74, 75
Cycle of organic nitrogen..... 5

PAGE
Daily analyses, at Chicago, etc. 41
Danube River, distribution of sewage in 116
Dedham tap-water, bacteria in 47
Deep wells 15
, bacteria in 17
Depths below surface, taking samples from 24
Desplaines River, B. coli in 89
Dextrose broth, for enrichment of B. coli 61
, method for isolation of B. coli 63
, preparation of 122
Dilution of samples in plating 28
Distribution of bacteria in sea-water 11
water 6
Diurnal variation in bacteria 14
Dogs, B. coli in 74, 75
Driven wells, bacteria in 40, 47
Dust in air 7

Egypt, cholera in 56
Elbe River, cholera bacilli in 55
Elsner's medium 54
Enrichment culture, for B. typhi 49
processes, disadvantage of 50
process, effect upon B. coli 50
Environment, effect of 14
Esmarch process 34
Examination of spring waters for B. coli 88

Feces, streptococci in 101
Fermentation, characteristic gas formula of B. coli 75
, gas formula of B. coli 95
of sugars by B. subtilis 45
, variations in 73
Field, plating in 34
Filter effluent, B. coli in 86, 87
Filtration of water 40
Fishes, B. coli in 75, 76
Food supply, effect of, upon bacteria 11, 14
Fowls, B. coli in 75
Fox River, B. coli in 90

PAGE
Framingham supply, bacteria in 42
Freezing of bacteria..... 12

Gas formula..... 95
Gas measurement, in fermentation tube..... 70
Gasometer..... 70
Gas production in dextrose broth by B. coli, time of..... 61
Gelatin. 119, 121
, liquefaction of..... 71
media..... 30
, physical condition of..... 30
Glycerin, agar, preparation of..... 122
Glycerin media 30
Goats, B. coli in 74, 75
Ground waters..... 15
, bacteria in 39
, bacteriological analysis of.....113, 114, 115
, character of bacteria in.....18, 39

Hamburg, cholera epidemic..... 55
Hartford supply, bacteria in..... 42
Hogs, B. coli in.....74, 75
Horses, B. coli in.....74, 75
Hudson River, B. coli in..... 83

Ice, bacteria in..... 12
Illinois River..... 11
, B. coli in..... 90
, distribution of sewage in..... 117
Incubation, conditions of..... 31
of agar plates at body temperature..... 44
, period of..... .....31, 32
Indol reaction..... 55
test 71
Interpretation of analyses..... 117
Interval between sampling and examination..... 28
Intestinal bacteria 99
Isar, River, bacteria in.....13, 14, 39
Isolation of B. coli, Chicago methods..... 89
, definition of species..... 67

PAGE
Isolation of B. coli, examination of subcultures..... 71
, from large samples 63, 64, 65
, growth on agar streak..... 67
, Mass. Inst. Tech. record blank..... 69
, Mass. State Board of Health methods 69
, necessity of standard methods..... 69
, percentage of cultures passing various tests..... 72
, period of preliminary enrichment..... 62
, phenol broth method..... 62
, preliminary enrichment 61
, separation of pure cultures..... 65
, significance of large samples.....87, 91
, significance of quantitative results..... 81
, variations in cultural reactions..... 73
streptococci..... 102

Jewell filter, bacterial efficiency 41

Kiel, bacteria in wells at.....15, 16

Lactic acid bacteria..... 79
Lactose-agar plate as a presumptive test 97
plates..... 44
, use in isolation of B. coli.....60, 65
, preparation of..... 122
broth, preparation of..... 122
, fermentation of, by B. coli.....58, 59
-fermenting bacteria in polluted waters..... 48
-fermenting organisms in normal water 48
Lake Champlain, bacteria in..... 10
of Lucerne, bacteria in..... 10
Zurich, bacteria in 38
Lakes, bacteria in..... 10
Lawrence city filter, bacterial efficiency of..... 40, 87
Leitmeritz, bacteria in wells at..... 15
Light, effect of, upon bacteria.....11, 13
in streams..... 13
Liquefying bacteria..... 108
, phenolated, for B. coli isolation..... 60
Litmus lactose agar plate, incubation of..... 66
plate, isolation of B. coli by..... 59

PAGE
Litmus lactose agar plate, technique of..... 45
, red colonies in..... 66
Litmus milk, preparation of..... 123
Liverpool tap-water, B. coli in..... 85
Loch Katrine, bacteria in..... 10
Loch of Lintralthen, bacteria in..... 10
Löeffler's blood serum..... 125
Lynn tap-water, bacteria in.....10, 47

MacConkey's media, preparation of..... 126
Mainz, bacteria in wells at..... 15
Maltose broth, preparation of..... 122
Manchester ship canal, B. coli in.....45, 85
Massachusetts Institute of Technology, blank for recording bacteriological analysis..... 69
Massachusetts State Board of Health 9, 72, 86, 88
, B. coli isolation..... 87
, bacteriological counts..... 40
, examination of springs..... 16
, methods for isolation of B. coli 69
Mechanical filtration..... 40
Medford tap-water, bacteria in.....10, 47
Media, alkaline, for isolation of Sp. choleræ..... 55
, bacteria developing on various..... 30
, comparability 20
, composition of..... 29
, limitations of ordinary..... 19
, percentage of bacteria developing on various..... 21
, practical requirements for..... 20
, reaction of..... 29
, standard methods.....119, 121
Merrimac River, bacteria in..... 9, 40
Metabolism of different classes of bacteria..... 37
Mice, B. coli in..... 74
Micro-organisms, effect of, upon bacteria..... 11, 12
Milk, preparation of..... 123
test..... 71
Milton tap-water bacteria in..... 47
Mississippi River, B. coli in..... 83, 90
Missouri River, B. coli in..... 90

PAGE
Mohawk River, B. coli in..... 83
Moisture in incubators..... 31
Montsouris, bacteria in air..... 8
Mud, colon bacilli in..... 45, 85
Multiplication of bacteria in spring waters..... 16, 25
in stored samples..... 25–27
Mur River, B. coli in..... 83

Nährstoff Heyden agar..... 19, 20, 21, 30
Naples, bacteria in sea-water at..... 10
Neumark, typhoid bacillus in well at..... 53
Neutral-red..... 97
broth, preparation of..... 126
Newburyport tap-water, bacteria in..... 47
Newport supply, bacteria in..... 42
New York Board of Health..... 82
, examination of rivers for B. coli..... 83
Nitrate solution, preparation of..... 124
test..... 71
, variations in..... 73
Nitrification..... 5
Nitrifying organisms..... 5
Nitrites..... 5
Nitrogen, cycle of organic..... 5
Normal waters, lactose fermenting bacteria in..... 48

Ohio River, B. coli in..... 85
, bacteria in..... 41
Organic matter, decomposition of..... 4, 111
in water, relation of bacteria to..... 37
, oxidation of..... 5
, relation of bacteria to..... 6
Osmotic pressure, effect of upon bacteria..... 11
Ourcq River, bacteria in..... 9
Overgrowth..... 62, 64
Oxidation of organic matter..... 5
Oxygen, effect of, upon bacterial counts..... 31

Para-colon organisms..... 78, 91, 92
Para-typhoid organisms..... 78
Parietti's solution..... 50

PAGE
Paris, bacteria in air of..... 8
Parma, B. coli in water supply of..... 78
in wells and springs near..... 78
Peabody tap-water, bacteria in..... 10, 47
Peptone method for isolation of cholera bacillus..... 56
solution, preparation of..... 124
test..... 71
Period of incubation..... 31
Phenol broth, composition of..... 62
, method for isolation of B. coli..... 62
, preparation of..... 125
Phenolated gelatin, use of, for typhoid isolation..... 50
lactose litmus agar..... 60
Plating..... 28
in the field..... 34
Plymouth tap-water, bacteria in..... 10, 47
Polluted waters, bacterial changes during storage..... 27
, blood temperature count..... 45
, lactose fermenting bacteria in..... 48
, specific pathogenes in..... 49
Ponds, bacteria in..... 10, 47
Pools, bacteria in..... 47
Potato, preparation of..... 124
gelatin, use for typhoid bacteria..... 50
Presumptive tests for B. coli..... 94 *et seq.*
Proteus organisms..... 108
Protozoa..... 12
Prussia, regulations regarding filter plants..... 41
Pseudocolon bacilli..... 68
Pump, sampling from..... 23
Pumping, effect of, upon bacterial content of well water..... 23

Quantitative analysis, arbitrary standards for..... 35
, composition of media..... 29
, conditions of incubation..... 31
, counting..... 33
, dilution..... 28
, general procedure..... 21
, interpretation of..... 35
, media for..... 19

PAGE
Quantitative analysis, period of incubation..... 31
, plating..... 28–29
, procedure for..... 21
, recording results..... 33
, sampling..... 21–24
, storage of samples..... 25
bacteriological analysis, significance of..... 111

Rabbits, B. coli in..... 74, 75
Rain, bacteria in..... 8, 47
, contamination of surface water by..... 9
Reaction of media..... 29
Recording quantitative results..... 33
Red colonies in litmus lactose agar..... 66
Relation of B. coli to B. acidi lactici..... 80
Rellingen, typhoid bacillus in well at..... 53

Saccharose broth, preparation of..... 122
Salem tap-water, bacteria in..... 10, 47
Sample bottles, cleaning of..... 22
, handling..... 22
, size of, effect of, upon multiplication of bacteria.... 27
, sterilization of..... 22
Sampling, for bacteriological analysis..... 21–25
, necessity for care in..... 37
Sand filtration..... 40
Sangamon River, B. coli in..... 90
Sanitary inspection..... 109
Research Laboratory, bacteria in Boston sewage..... 45
Sea-water, bacteria in..... 10
Seasonal distribution of bacteria in surface waters..... 9
variation of bacterial content in surface waters.....9, 38
Sedimentation of bacteria..... 11
Seine, bacteria in..... 39
Self-purification of lakes and ponds..... 10
of streams..... 8, 13, 39, 113
Severn River, colon bacilli in..... 45, 85
Sewage, B. coli in..... 89
, bacteria in..... 45
, distribution of, in water..... 116, 117
pollution, bacteria in relation to..... 39

PAGE
Sewage, streptococci in..... 62, 100, 101
, typhoid bacillus in..... 51
Shallow wells, bacteria in..... 15, 16
Sheep, B. coli in..... 75
Shiga bacillus..... 53
Significance of B. coli..... 89
in water.....76 *et seq.*, 93
, necessity for quantitative results.... 81
of blood temperature count..... 43
of quantitative analysis..... 38
of streptococci in water..... 105
Snow, bacteria in..... 8, 47
Sodium taurocholate medium..... 98
Specific pathogenes in polluted water..... 49
Spirillum choleræ in Elbe River..... 55
, isolation of..... 54, 55
of Asiatic cholera, isolation from polluted water..... 49
Spore-forming pathogenes in water, isolation of..... 56
Spring waters, multiplication of bacteria in..... 16
Springs, bacteria in..... 15, 16, 47
Standard for interpreting presumptive test..... 97
Sterilization of gelatine, effect of..... 30
sample bottles..... 22
Storage..... 11
, effect on sample..... 37
, multiplication of bacteria during..... 25–27
of polluted waters..... 27
of samples, allowable maximum..... 28
Strassburg, B. coli in water of..... 76
Streams, bacteria in..... 39
, sedimentation of bacteria in..... 11
Streams, self-purification of..... 8, 13
Streptococci as indicative of pollution..... 100
, colonies on litmus lactose agar..... 66
, cultural characters..... 99
, decomposition of sugars by..... 44
, detection of, in presence of B. coli..... 104
, growth on agar streak..... 67
, habitat of..... 101

PAGE
Streptococci in feces..... 101
in polluted waters..... 92, 102
in sewage..... 101
in sewage polluted rivers..... 100
in stored sewage..... 101
in unpolluted water..... 91, 101
, isolation of..... 102
, overgrowth of B. coli by..... 102, 104
, relation to B. coli..... 102, 103, 104
sewage pollution..... 105
, sewage..... 62, 100
, significance of..... 112
, types of colonies on litmus lactose agar..... 66
Streptococcus erysipelatos..... 99
Sudbury River, chemical and bacteriological examination..... 113
Sugar broth, preparation of..... 122
Sugars, decomposition of..... 44
Surface wash, bacteria in..... 8
water, sampling of..... 24
, bacteria in..... 9, 39, 47

Tap-water sampling..... 22
Taunton tap-water, bacteria in..... 10, 47
Temperature, effect of, upon bacteria..... 11, 12
, effect upon multiplication of bacteria in stored samples, 26, 27
of incubation..... 31
Thames River, bacteria in..... 9
Titration of media..... 120
Toronto supply, bacteria in..... 41
Toxic products..... 12
Turin, B. coli in unpolluted waters near..... 78
Turkeys, B. coli in..... 75
Typhoid bacillus in ice..... 12
in sewage..... 57
in well at Neumark..... 53
Rellingen..... 54
isolation from polluted water..... 49
, reported isolation from water..... 53
fever, spring epidemics..... 38

PAGE

Urea, decomposition of..... 5

Value of isolated analyses..... 41
Variation of B. coli..... 68
Variations in cultural reactions..... 73

Wakefield and Stoneham tap-water, bacteria in..... 10,
Water, B. coli in different grades of..... 96
in polluted and unpolluted..... 63
bacteria, peculiar metabolism of..... 37
, bacteriological analysis of..... 69
, chemical composition, relation of bacteria to..... 37, 38
, chemical examination of 110
, complete bacteriological analysis..... 112
, distribution of bacteria in..... 6
purification..... 40
supplies, sanitary inspection of..... 109
Waters, bacteria in various classes of..... 21
, classification of..... 16
Well, typhoid bacillus in, at Neumark..... 53
at Rellingen..... 53
water, bacteria in..... 42
Wells, bacteria in..... 48
Westerly tap-water, bacteria in..... 47
Widal reaction..... 53
Wood's Hole, bacteria in sea-water at..... 110
Wurtz litmus lactose agar..... 59

SHORT-TITLE CATALOGUE

OF THE

PUBLICATIONS

OF

JOHN WILEY & SONS,

NEW YORK.

LONDON: CHAPMAN & HALL, LIMITED.

ARRANGED UNDER SUBJECTS.

Descriptive circulars sent on application. Books marked with an asterisk are sold at *net* prices only, a double asterisk (**) books sold under the rules of the American Publishers' Association at *net* prices subject to an extra charge for postage. All books are bound in cloth unless otherwise stated.

AGRICULTURE.

Armsby's Manual of Cattle-feeding.....12mo, $1 75
Principles of Animal Nutrition.....8vo, 4 00
Budd and Hansen's American Horticultural Manual:
Part I.—Propagation, Culture, and Improvement.....12mo, 1 50
Part II.—Systematic Pomology.....12mo, 1 50
Downing's Fruits and Fruit-trees of America.....8vo, 5 00
Elliott's Engineering for Land Drainage.....12mo, 1 50
Practical Farm Drainage.....12mo, 1 00
Green's Principles of American Forestry.....12mo, 1 50
Grotenfelt's Principles of Modern Dairy Practice. (Woll.).....12mo, 2 00
Kemp's Landscape Gardening.....12mo, 2 50
Maynard's Landscape Gardening as Applied to Home Decoration.....12mo, 1 50
Sanderson's Insects Injurious to Staple Crops.....12mo, 1 50
Insects Injurious to Garden Crops. (*In preparation.*)
Insects Injuring Fruits. (*In preparation.*)
Stockbridge's Rocks and Soils.....8vo, 2 50
Woll's Handbook for Farmers and Dairymen.....16mo, 1 50

ARCHITECTURE.

Baldwin's Steam Heating for Buildings.....12mo, 2 50
Berg's Buildings and Structures of American Railroads.....4to, 5 00
Birkmire's Planning and Construction of American Theatres..... 8vo, 3 00
Architectural Iron and Steel.....8vo, 3 50
Compound Riveted Girders as Applied in Buildings.....8vo, 2 00
Planning and Construction of High Office Buildings.....8vo, 3 50
Skeleton Construction in Buildings.....8vo, 3 00
Briggs's Modern American School Buildings.....8vo, 4 00
Carpenter's Heating and Ventilating of Buildings.....8vo, 4 00
Freitag's Architectural Engineering. 2d Edition, Rewritten.....8vo, 3 50
Fireproofing of Steel Buildings.....8vo, 2 50
French and Ives's Stereotomy.....8vo, 2 50
Gerhard's Guide to Sanitary House-inspection.....16mo, 1 00
Theatre Fires and Panics.....12mo 1 50

Holly's Carpenters' and Joiners' Handbook.....18mo, 0 75
Johnson's Statics by Algebraic and Graphic Methods.....8vo 2 00
Kidder's Architect's and Builder's Pocket-book. (*Rewritten edition in preparation.*)
Merrill's Stones for Building and Decoration.....8vo, 5 00
Monckton's Stair-building.....4to, 4 00
Patton's Practical Treatise on Foundations.....8vo, 5 00
Siebert and Biggin's Modern Stone-cutting and Masonry.....8vo, 1 50
Snow's Principal Species of Wood.....8vo, 3 50
Sondericker's Graphic Statics with Applications to Trusses, Beams, and Arches. 8vo, 2 00
Wait's Engineering and Architectural Jurisprudence.....8vo, 6 00
Sheep, 6 50
Law of Operations Preliminary to Construction in Engineering and Architecture.....8vo, 5 00
Sheep, 5 50
Law of Contracts.....8vo, 3 00
Woodbury's Fire Protection of Mills.....8vo, 2 50
Worcester and Atkinson's Small Hospitals, Establishment and Maintenance, Suggestions for Hospital Architecture, with Plans for a Small Hospital. 12mo, 1 25
The World's Columbian Exposition of 1893.....Large 4to, 1 00

ARMY AND NAVY.

Bernadou's Smokeless Powder, Nitro-cellulose, and the Theory of the Cellulose Molecule.....12mo, 2 50
* Bruff's Text-book Ordnance and Gunnery.....8vo, 6 00
Chase's Screw Propellers and Marine Propulsion.....8vo, 3 00
Craig's Azimuth.....4to, 3 50
Cremore and Squire's Polarizing Photo-chronograph.....8vo, 3 00
Cronkhite's Gunnery for Non-commissioned Officers.....24mo, morocco, 2 00
* Davis's Elements of Law.....8vo, 2 50
* Treatise on the Military Law of United States.....8vo, 7 00
Sheep, 7 50
De Brack's Cavalry Outpost Duties. (Carr.).....24mo morocco, 2 00
Dietz's Soldier's First Aid Handbook.....16mo, morocco, 1 25
* Dredge's Modern French Artillery.....4to, half morocco, 15 00
Durand's Resistance and Propulsion of Ships.....8vo, 5 00
* Dyer's Handbook of Light Artillery.....12mo, 3 00
Eissler's Modern High Explosives.....8vo, 4 00
* Fiebeger's Text-book on Field Fortification.....Small 8vo, 2 00
Hamilton's The Gunner's Catechism.....18mo, 1 00
* Hoff's Elementary Naval Tactics.....8vo, 1 50
Ingalls's Handbook of Problems in Direct Fire.....8vo, 4 00
* Ballistic Tables.....8vo, 1 50
* Lyons's Treatise on Electromagnetic Phenomena. Vols. I. and II..8vo, each, 6 00
* Mahan's Permanent Fortifications. (Mercur.).....8vo, half morocco, 7 50
Manual for Courts-martial.....16mo, morocco, 1 50
* Mercur's Attack of Fortified Places.....12mo, 2 00
* Elements of the Art of War.....8vo, 4 00
Metcalf's Cost of Manufactures—And the Administration of Workshops, Public and Private.....8vo, 5 00
* Ordnance and Gunnery.....12mo, 5 00
Murray's Infantry Drill Regulations.....18mo, paper, 10
* Phelps's Practical Marine Surveying.....8vo, 2 50
Powell's Army Officer's Examiner.....12mo, 4 00
Sharpe's Art of Subsisting Armies in War.....18mo, morocco, 1 50

* Walke's Lectures on Explosives.....8vo, 4 00
* Wheeler's Siege Operations and Military Mining.....8vo, 2 00
Winthrop's Abridgment of Military Law.....12mo, 2 50
Woodhull's Notes on Military Hygiene.....16mo, 1 50
Young's Simple Elements of Navigation.....16mo, morocco, 1 00
Second Edition, Enlarged and Revised.....16mo, morocco, 2 00

ASSAYING.

Fletcher's Practical Instructions in Quantitative Assaying with the Blowpipe. 12mo, morocco, 1 50
Furman's Manual of Practical Assaying.....8vo, 3 00
Miller's Manual of Assaying.....12mo, 1 00
O'Driscoll's Notes on the Treatment of Gold Ores.....8vo, 2 00
Ricketts and Miller's Notes on Assaying.....8vo, 3 00
Ulke's Modern Electrolytic Copper Refining.....8vo, 3 00
Wilson's Cyanide Processes.....12mo, 1 50
Chlorination Process.....12mo, 1 50

ASTRONOMY.

Comstock's Field Astronomy for Engineers.....8vo, 2 50
Craig's Azimuth.....4to, 3 50
Doolittle's Treatise on Practical Astronomy.....8vo, 4 00
Gore's Elements of Geodesy.....8vo, 2 50
Hayford's Text-book of Geodetic Astronomy.....8vo, 3 00
Merriman's Elements of Precise Surveying and Geodesy.....8vo, 2 50
* Michie and Harlow's Practical Astronomy.....8vo, 3 00
* White's Elements of Theoretical and Descriptive Astronomy.....12mo, 2 00

BOTANY.

Davenport's Statistical Methods, with Special Reference to Biological Variation. 16mo, morocco, 1 25
Thomé and Bennett's Structural and Physiological Botany.....16mo, 2 25
Westermaier's Compendium of General Botany. (Schneider.).....8vo, 2 00

CHEMISTRY.

Adriance's Laboratory Calculations and Specific Gravity Tables.....12mo, 1 25
Allen's Tables for Iron Analysis.....8vo, 3 00
Arnold's Compendium of Chemistry. (Mandel.) (*In preparation.*)
Austen's Notes for Chemical Students.....12mo, 1 50
Bernadou's Smokeless Powder.—Nitro-cellulose, and Theory of the Cellulose Molecule.....12mo, 2 50
Bolton's Quantitative Analysis.....8vo, 1 50
* Browning's Introduction to the Rarer Elements.....8vo, 1 50
Brush and Penfield's Manual of Determinative Mineralogy.....8vo, 4 00
Classen's Quantitative Chemical Analysis by Electrolysis. (Boltwood.)... 8vo, 3 00
Cohn's Indicators and Test-papers.....12mo, 2 00
Tests and Reagents.....8vo, 3 00
Copeland's Manual of Bacteriology. (*In preparation.*)
Craft's Short Course in Qualitative Chemical Analysis. (Schaeffer.)....12mo, 1 50
Drechsel's Chemical Reactions. (Merrill.).....12mo, 1 25
Duhem's Thermodynamics and Chemistry. (Burgess.).....8vo, 4 00
Eissler's *Modern High Explosives*.....8vo, 4 00

Effront's Enzymes and their Applications. (Prescott.).....8vo, 3 00
Erdmann's Introduction to Chemical Preparations. (Dunlap.).....12mo, 1 25
Fletcher's Practical Instructions in Quantitative Assaying with the Blowpipe. 12mo, morocco, 1 50
Fowler's Sewage Works Analyses.....12mo, 2 00
Fresenius's Manual of Qualitative Chemical Analysis. (Wells.).....8vo, 5 00
Manual of Qualitative Chemical Analysis. Part I. Descriptive. (Wells.) 8vo, 3 00
System of Instruction in Quantitative Chemical Analysis. (Cohn.) 2 vols. (*Shortly.*)
Fuertes's Water and Public Health.....12mo, 1 50
Furman's Manual of Practical Assaying.....8vo, 3 00
Gill's Gas and Fuel Analysis for Engineers.....12mo, 1 25
Grotenfelt's Principles of Modern Dairy Practice. (Woll.).....12mo. 2 00
Hammarsten's Text-book of Physiological Chemistry. (Mandel.).....8vo, 4 00
Helm's Principles of Mathematical Chemistry. (Morgan.).....12mo, 1 50
Hinds's Inorganic Chemistry.....8vo, 3 00
* Laboratory Manual for Students.....12mo, 75
Holleman's Text-book of Inorganic Chemistry. (Cooper.).....8vo, 2 50
Text-book of Organic Chemistry. (Walker and Mott.).....8vo, 2 50
Hopkins's Oil-chemists' Handbook.....8vo, 3 00
Jackson's Directions for Laboratory Work in Physiological Chemistry..8vo, 1 00
Keep's Cast Iron.....8vo, 2 50
Ladd's Manual of Quantitative Chemical Analysis.....12mo 1 00
Landauer's Spectrum Analysis. (Tingle.).....8vo, 3 00
Lassar-Cohn's Practical Urinary Analysis. (Lorenz.).....12mo, 1 00
Leach's The Inspection and Analysis of Food with Special Reference to State Control. (*In preparation.*)
Löb's Electrolysis and Electrosynthesis of Organic Compounds. (Lorenz.) 12mo, 1 00
Mandel's Handbook for Bio-chemical Laboratory.....12mo, 1 50
* Martin's Laboratory Guide to Qualitative Analysis with the Blowpipe..12mo, 60
Mason's Water-supply. (Considered Principally from a Sanitary Standpoint.) 3d Edition, Rewritten.....8vo, 4 00
Examination of Water. (Chemical and Bacteriological.).....12mo, 1 25
Meyer's Determination of Radicles in Carbon Compounds. (Tingle.)..12mo, 1 00
Miller's Manual of Assaying.....12mo, 1 00
Mixter's Elementary Text-book of Chemistry.....12mo, 1 50
Morgan's Outline of Theory of Solution and its Results.....12mo, 1 00
Elements of Physical Chemistry.....12mo, 2 00
Nichols's Water-supply. (Considered mainly from a Chemical and Sanitary Standpoint, 1883.).....8vo, 2 50
O'Brine's Laboratory Guide in Chemical Analysis.....8vo, 2 00
O'Driscoll's Notes on the Treatment of Gold Ores.....8vo, 2 00
Ost and Kolbeck's Text-book of Chemical Technology. (Lorenz—Bozart.) (*In preparation.*)
* Penfield's Notes on Determinative Mineralogy and Record of Mineral Tests. 8vo, paper, 50
Pictet's The Alkaloids and their Chemical Constitution. (Biddle.) (*In preparation.*)
Pinner's Introduction to Organic Chemistry. (Austen.).....12mo, 1 50
Poole's Calorific Power of Fuels.....8vo, 3 00
* Reisig's Guide to Piece-dyeing.....8vo, 25 00
Richards and Woodman's Air, Water, and Food from a Sanitary Standpoint.8vo, 2 00
Richards's Cost of Living as Modified by Sanitary Science.....12mo, 1 00
Cost of Food a Study in Dietaries.....12mo, 1 00
* Richards and Williams's The Dietary Computer.....8vo, 1 50
Ricketts and Russell's Skeleton Notes upon Inorganic Chemistry. (Part I.—Non-metallic Elements.).....8vo, morocco, 75

Ricketts and Miller's Notes on Assaying.....8vo, 3 00
Rideal's Sewage and the Bacterial Purification of Sewage.....8vo, 3 50
Ruddiman's Incompatibilities in Prescriptions.....8vo, 2 00
Salkowski's Physiological and Pathological Chemistry. (Orndorff.)
(*Shortly.*)
Schimpf's Text-book of Volumetric Analysis.....12mo, 2 50
Essentials of Volumetric Analysis.....12mo, 1 25
Spencer's Handbook for Chemists of Beet-sugar Houses.....16mo, morocco, 3 00
Handbook for Sugar Manufacturers and their Chemists..16mo, morocco, 2 00
Stockbridge's Rocks and Soils.....8vo, 2 50
* Tillman's Elementary Lessons in Heat.....8vo, 1 50
* Descriptive General Chemistry.....8vo 3 00
Treadwell's Qualitative Analysis. (Hall.).....8vo, 3 00
Turneaure and Russell's Public Water-supplies.....8vo, 5 00
Van Deventer's Physical Chemistry for Beginners. (Boltwood.).....12mo, 1 50
* Walke's Lectures on Explosives.....8vo, 4 00
Wells's Laboratory Guide in Qualitative Chemical Analysis.....8vo, 1 50
Short Course in Inorganic Qualitative Chemical Analysis for Engineering Students.....12mo, 1 50
Whipple's Microscopy of Drinking-water.....8vo, 3 50
Wiechmann's Sugar Analysis.....Small 8vo, 2 50
Wilson's Cyanide Processes.....12mo, 1 50
Chlorination Process.....12mo 1 50
Wulling's Elementary Course in Inorganic Pharmaceutical and Medical Chemistry.....12mo 2 00

CIVIL ENGINEERING.

BRIDGES AND ROOFS. HYDRAULICS MATERIALS OF ENGINEERING RAILWAY ENGINEERING.

Baker's Engineers' Surveying Instruments.....12mo, 3 00
Bixby's Graphical Computing Table.....Paper 19¼×24¼ inches 25
** Burr's Ancient and Modern Engineering and the Isthmian Canal. (Postage, 27 cents additional.).....8vo, net, 3 50
Comstock's Field Astronomy for Engineers.....8vo, 2 50
Davis's Elevation and Stadia Tables.....8vo, 1 00
Elliott's Engineering for Land Drainage.....12mo, 1 50
Practical Farm Drainage.....12mo, 1 00
Folwell's Sewerage. (Designing and Maintenance.).....8vo, 3 00
Freitag's Architectural Engineering. 2d Edition, Rewritten.....8vo, 3 50
French and Ives's Stereotomy.....8vo, 2 50
Goodhue's Municipal Improvements.....12mo, 1 75
Goodrich's Economic Disposal of Towns' Refuse.....8vo, 3 50
Gore's Elements of Geodesy.....8vo, 2 50
Hayford's Text-book of Geodetic Astronomy.....8vo, 3 00
Howe's Retaining Walls for Earth.....12mo, 1 25
Johnson's Theory and Practice of Surveying.....Small 8vo, 4 00
Statics by Algebraic and Graphic Methods.....8vo, 2 00
Kiersted's Sewage Disposal.....12mo, 1 25
Laplace's Philosophical Essay on Probabilities. (Truscott and Emory.) 12mo, 2 00
Mahan's Treatise on Civil Engineering. (1873.) (Wood.).....8vo, 5 00
* Descriptive Geometry.....8vo, 1 50
Merriman's Elements of Precise Surveying and Geodesy.....8vo, 2 50
Elements of Sanitary Engineering.....8vo, 2 00
Merriman and Brooks's Handbook for Surveyors.....16mo, morocco, 2 00
Nugent's Plane Surveying.....8vo, 3 50
Ogden's Sewer Design.....12mo, 2 00
Patton's Treatise on Civil Engineering.....8vo half leather, 7 50

Thurston's Materials of Engineering. 3 Parts..... 8vo, 8 00
Part I.—Non-metallic Materials of Engineering and Metallurgy.....8vo, 2 00
Part II.—Iron and Steel.....8vo, 3 50
Part III.—A Treatise on Brasses, Bronzes, and Other Alloys and their Constituents..... 8vo, 2 50
Thurston's Text-book of the Materials of Construction 8vo, 5 00
Tillson's Street Pavements and Paving Materials.....8vo, 4 00
Waddell's De Pontibus. (A Pocket-book for Bridge Engineers.)..16mo, mor., 3 00
Specifications for Steel Bridges.....12mo, 1 25
Wood's Treatise on the Resistance of Materials, and an Appendix on the Preservation of Timber 8vo, 2 00
Elements of Analytical Mechanics.....8vo, 3 00
Wood's Rustless Coatings. (*Shortly.*)

RAILWAY ENGINEERING.

Andrews's Handbook for Street Railway Engineers. 3×5 inches. morocco, 1 25
Berg's Buildings and Structures of American Railroads.....4to, 5 00
Brooks's Handbook of Street Railroad Location.....16mo. morocco, 1 50
Butts's Civil Engineer's Field-book.....16mo, morocco, 2 50
Crandall's Transition Curve.....16mo, morocco, 1 50
Railway and Other Earthwork Tables.....8vo, 1 50
Dawson's "Engineering" and Electric Traction Pocket-book. 16mo, morocco, 5 00
Dredge's History of the Pennsylvania Railroad: (1879).....Paper, 5 00
* Drinker's Tunneling, Explosive Compounds, and Rock Drills, 4to, half mor., 25 00
Fisher's Table of Cubic Yards.....Cardboard 25
Godwin's Railroad Engineers' Field-book and Explorers' Guide.....16mo, mor., 2 50
Howard's Transition Curve Field-book.....16mo, morocco. 1 50
Hudson's Tables for Calculating the Cubic Contents of Excavations and Embankments 8vo, 1 00
Molitor and Beard's Manual for Resident Engineers.....16mo, 1 00
Nagle's Field Manual for Railroad Engineers.....16mo, morocco. 3 00
Philbrick's Field Manual for Engineers.....16mo, morocco, 3 00
Pratt and Alden's Street-railway Road-bed.....8vo, 2 00
Searles's Field Engineering.....16mo, morocco, 3 00
Railroad Spiral.....16mo, morocco, 1 50
Taylor's Prismoidal Formulæ and Earthwork.....8vo, 1 50
* Trautwine's Method of Calculating the Cubic Contents of Excavations and Embankments by the Aid of Diagrams.....8vo, 2 00
The Field Practice of Laying Out Circular Curves for Railroads. 12mo, morocco, 2 50
* Cross-section Sheet.....Paper, 25
Webb's Railroad Construction. 2d Edition, Rewritten.....16mo. morocco, 5 00
Wellington's Economic Theory of the Location of Railways.....Small 8vo, 5 00

DRAWING.

Barr's Kinematics of Machinery.....8vo, 2 50
* Bartlett's Mechanical Drawing.....8vo, 3 00
* " " " Abridged Ed.....8vo, 1 50
Coolidge's Manual of Drawing..... 8vo, paper, 1 00
Durley's Kinematics of Machines.....8vo, 4 00
Hill's Text-book on Shades and Shadows, and Perspective.....8vo, 2 00
Jones's Machine Design:
Part I.—Kinematics of Machinery.....8vo, 1 50
Part II.—Form, Strength, and Proportions of Parts.....8vo, 3 00

MacCord's Elements of Descriptive Geometry.....8vo, 3 00
Kinematics; or, Practical Mechanism.....8vo, 5 00
Mechanical Drawing.....4to, 4 00
Velocity Diagrams.....8vo, 1 50
* Mahan's Descriptive Geometry and Stone-cutting.....8vo, 1 50
Industrial Drawing. (Thompson.).....8vo, 3 50
Reed's Topographical Drawing and Sketching.....4to, 5 00
Reid's Course in Mechanical Drawing.....8vo, 2 00
Text-book of Mechanical Drawing and Elementary Machine Design..8vo, 3 00
Robinson's Principles of Mechanism.....8vo, 3 00
Smith's Manual of Topographical Drawing. (McMillan.).....8vo, 2 50
Warren's Elements of Plane and Solid Free-hand Geometrical Drawing..12mo, 1 00
Drafting Instruments and Operations.....12mo, 1 25
Manual of Elementary Projection Drawing.....12mo, 1 50
Manual of Elementary Problems in the Linear Perspective of Form and Shadow.....12mo, 1 00
Plane Problems in Elementary Geometry.....12mo, 1 25
Primary Geometry.....12mo, 75
Elements of Descriptive Geometry, Shadows, and Perspective.....8vo, 3 50
General Problems of Shades and Shadows.....8vo, 3 00
Elements of Machine Construction and Drawing.....8vo, 7 50
Problems, Theorems, and Examples in Descriptive Geometry.....8vo, 2 50
Weisbach's Kinematics and the Power of Transmission. (Hermann and Klein.).....8vo, 5 00
Whelpley's Practical Instruction in the Art of Letter Engraving.....12mo, 2 00
Wilson's Topographic Surveying.....8vo, 3 50
Free-hand Perspective.....8vo, 2 50
Free-hand Lettering.....8vo, 1 00
Woolf's Elementary Course in Descriptive Geometry.....Large 8vo, 3 00

ELECTRICITY AND PHYSICS.

Anthony and Brackett's Text-book of Physics. (Magie.).....Small 8vo, 3 00
Anthony's Lecture-notes on the Theory of Electrical Measurements.....12mo, 1 00
Benjamin's History of Electricity.....8vo, 3 00
Voltaic Cell.....8vo, 3 00
Classen's Quantitative Chemical Analysis by Electrolysis. (Boltwood.)..8vo, 3 00
Crehore and Squier's Polarizing Photo-chronograph.....8vo, 3 00
Dawson's "Engineering" and Electric Traction Pocket-book..16mo, morocco, 5 00
Dolezalek's Theory of the Lead Accumulator. (Storage Battery.) (*Shortly.*) (Von Ende.)
Duhem's Thermodynamics and Chemistry. (Burgess.).....8vo, 4 00
Flather's Dynamometers, and the Measurement of Power.....12mo, 3 00
Gilbert's De Magnete. (Mottelay.).....8vo, 2 50
Hanchett's Alternating Currents Explained. (*Shortly.*)
Holman's Precision of Measurements.....8vo, 2 00
Telescopic Mirror-scale Method, Adjustments, and Tests.....Large 8vo, 75
Landauer's Spectrum Analysis. (Tingle.).....8vo, 3 00
Le Chatelier's High-temperature Measurements. (Boudouard—Burgess.)12mo, 3 00
Löb's Electrolysis and Electrosynthesis of Organic Compounds. (Lorenz.) 12mo, 1 00
* Lyons's Treatise on Electromagnetic Phenomena. Vols. I. and II. 8vo, each, 6 00
* Michie. Elements of Wave Motion Relating to Sound and Light.....8vo, 4 00
Niaudet's Elementary Treatise on Electric Batteries. (Fishback.).....12mo, 2 50
* Parshall and Hobart's Electric Generators.....Small 4to. half morocco, 10 00
* Rosenberg's Electrical Engineering. (Haldane Gee—Kinzbrunner.)....8vo, 1 50
Ryan, Norris, and Hoxie's Electrical Machinery. Vol. I.....8vo, 2 50
Thurston's Stationary Steam-engines.....8vo, 2 50
* Tillman's Elementary Lessons in Heat.....8vo, 1 50

Tory and Pitcher's Manual of Laboratory Physics ..... Small 8vo, 2 00
Ulke's Modern Electrolytic Copper Refining 8vo, 3 00

LAW.

* Davis's Elements of Law 8vo, 2 50
* Treatise on the Military Law of United States 8vo, 7 00
* Sheep, 7 50
Manual for Courts-martial ..... 16mo, morocco, 1 50
Wait's Engineering and Architectural Jurisprudence 8vo, 6 00
Sheep, 6 50
Law of Operations Preliminary to Construction in Engineering and Architecture 8vo, 5 00
Sheep, 5 50
Law of Contracts 8vo, 3 00
Winthrop's Abridgment of Military Law 12mo, 2 50

MANUFACTURES.

Bernadou's Smokeless Powder—Nitro-cellulose and Theory of the Cellulose Molecule 12mo, 2 50
Bolland's Iron Founder 12mo, 2 50
"The Iron Founder," Supplement 12mo, 2 50
Encyclopedia of Founding and Dictionary of Foundry Terms Used in the Practice of Moulding 12mo, 3 00
Eissler's Modern High Explosives 8vo, 4 00
Effront's Enzymes and their Applications. (Prescott.) 8vo, 3 00
Fitzgerald's Boston Machinist 18mo, 1 00
Ford's Boiler Making for Boiler Makers 18mo, 1 00
Hopkins's Oil-chemists' Handbook 8vo, 3 00
Keep's Cast Iron 8vo, 2 50
Leach's The Inspection and Analysis of Food with Special Reference to State Control. (*In preparation.*)
Metcalf's Steel. A Manual for Steel-users 12mo, 2 00
Metcalfe's Cost of Manufactures—And the Administration of Workshops, Public and Private 8vo, 5 00
Meyer's Modern Locomotive Construction 4to, 10 00
* Reisig's Guide to Piece-dyeing 8vo, 25 00
Smith's Press-working of Metals 8vo, 3 00
Wire: Its Use and Manufacture ..... Small 4to, 3 00
Spalding's Hydraulic Cement 12mo, 2 00
Spencer's Handbook for Chemists of Beet-sugar Houses ..... 16mo, morocco, 3 00
Handbook for Sugar Manufacturers and their Chemists ..... 16mo, morocco, 2 00
Thurston's Manual of Steam-boilers, their Designs, Construction and Operation 8vo, 5 00
* Walke's Lectures on Explosives 8vo, 4 00
West's American Foundry Practice 12mo, 2 50
Moulder's Text-book 12mo, 2 50
Wiechmann's Sugar Analysis ..... Small 8vo, 2 50
Wolff's Windmill as a Prime Mover 8vo, 3 00
Woodbury's Fire Protection of Mills 8vo, 2 50

MATHEMATICS.

Baker's Elliptic Functions 8vo, 1 50
* *Bass's Elements of Differential Calculus* 12mo, 4 00
Briggs's Elements of Plane Analytic Geometry 12mo, 1 00

Compton's Manual of Logarithmic Computations.....12mo, 1 50
Davis's Introduction to the Logic of Algebra.....8vo, 1 50
* Dickson's College Algebra ..... Large 12mo, 1 50
* Introduction to the Theory of Algebraic Equations ..... Large 12mo, 1 25
Halsted's Elements of Geometry 8vo, 1 75
Elementary Synthetic Geometry.....8vo. 1 50
Rational Geometry. (*Shortly.*)
* Johnson's Three-place Logarithmic Tables: Vest-pocket size.....paper, 15
100 copies for 5 00
* Mounted on heavy cardboard, 8 × 10 inches, 25
10 copies for 2 00
Elementary Treatise on the Integral Calculus.....Small 8vo, 1 50
Curve Tracing in Cartesian Co-ordinates.....12mo, 1 00
Treatise on Ordinary and Partial Differential Equations.....Small 8vo, 3 50
Theory of Errors and the Method of Least Squares.....12mo, 1 50
* Theoretical Mechanics.....12mo, 3 00
Laplace's Philosophical Essay on Probabilities. (Truscott and Emory.) 12mo, 2 00
* Ludlow and Bass. Elements of Trigonometry and Logarithmic and Other Tables.....8vo, 3 00
Trigonometry and Tables published separately..... Each, 2 00
Maurer's Technical Mechanics.....8vo, 4 00
Merriman and Woodward's Higher Mathematics 8vo, 5 00
Merriman's Method of Least Squares.....8vo, 2 00
Rice and Johnson's Elementary Treatise on the Differential Calculus.Sm., 8vo, 3 00
Differential and Integral Calculus. 2 vols. in one.....Small 8vo, 2 50
Wood's Elements of Co-ordinate Geometry.....8vo, 2 00
Trigonometry: Analytical, Plane, and Spherical.....12mo, 1 00

MECHANICAL ENGINEERING.

MATERIALS OF ENGINEERING, STEAM-ENGINES AND BOILERS.

Baldwin's Steam Heating for Buildings.....12mo, 2 50
Barr's Kinematics of Machinery.....8vo, 2 50
* Bartlett's Mechanical Drawing.....8vo, 3 00
* " " " Abridged Ed.....8vo, 1 50
Benjamin's Wrinkles and Recipes.....12mo, 2 00
Carpenter's Experimental Engineering.....8vo, 6 00
Heating and Ventilating Buildings.....8vo, 4 00
Cary's Smoke Suppression in Plants using Bituminous Coal. (*In preparation.*)
Clerk's Gas and Oil Engine.....Small 8vo, 4 00
Coolidge's Manual of Drawing.....8vo, paper, 1 00
Cromwell's Treatise on Toothed Gearing.....12mo, 1 50
Treatise on Belts and Pulleys.....12mo, 1 50
Durley's Kinematics of Machines.....8vo, 4 00
Flather's Dynamometers and the Measurement of Power.....12mo, 3 00
Rope Driving.....12mo, 2 00
Gill's Gas and Fuel Analysis for Engineers.....12mo, 1 25
Hall's Car Lubrication. 12mo, 1 00
Hutton's The Gas Engine.....8vo, 5 00
Jones's Machine Design:
Part I.—Kinematics of Machinery 8vo, 1 50
Part II.—Form, Strength, and Proportions of Parts.....8vo, 3 00
Kent's Mechanical Engineer's Pocket-book.....16mo, morocco, 5 00
Kerr's Power and Power Transmission.....8vo, 2 00
MacCord's Kinematics; or, Practical Mechanism.....8vo, 5 00
Mechanical Drawing.....4to, 4 00
Velocity Diagrams. 8vo, 1 50

Mahan's Industrial Drawing. (Thompson.)....8vo, 3 50
Poole's Calorific Power of Fuels....8vo, 3 00
Reid's Course in Mechanical Drawing....8vo. 2 00
Text-book of Mechanical Drawing and Elementary Machine Design..8vo, 3 00
Richards's Compressed Air....12mo, 1 50
Robinson's Principles of Mechanism....8vo, 3 00
Smith's Press-working of Metals....8vo, 3 00
Thurston's Treatise on Friction and Lost Work in Machinery and Mul Work....8vo, 3 00
Animal as a Machine and Prime Motor, and the Laws of Energetics.12mo, 1 00
Warren's Elements of Machine Construction and Drawing....8vo, 7 50
Weisbach's Kinematics and the Power of Transmission. Herrmann—Klein.)....8vo, 5 00
Machinery of Transmission and Governors. (Herrmann—Klein.)..8vo, 5 00
Hydraulics and Hydraulic Motors. (Du Bois.)....8vo, 5 00
Wolff's Windmill as a Prime Mover....8vo, 3 00
Wood's Turbines....8vo, 2 50

MATERIALS OF ENGINEERING.

Bovey's Strength of Materials and Theory of Structures....8vo, 7 50
Burr's Elasticity and Resistance of the Materials of Engineering. 6th Edition, Reset....8vo. 7 50
Church's Mechanics of Engineering....8vo, 6 00
Johnson's Materials of Construction....Large 8vo, 6 00
Keep's Cast Iron....8vo, 2 50
Lanza's Applied Mechanics....8vo, 7 50
Martens's Handbook on Testing Materials. (Henning.)....8vo, 7 50
Merriman's Text-book on the Mechanics of Materials....8vo, 4 00
Strength of Materials....12mo, 1 00
Metcalf's Steel. A Manual for Steel-users....12mo 2 00
Smith's Wire: Its Use and Manufacture....Small 4to, 3 00
Materials of Machines....12mo 1 00
Thurston's Materials of Engineering....3 vols., 8vo, 8 00
Part II.—Iron and Steel....8vo, 3 50
Part III.—A Treatise on Brasses, Bronzes, and Other Alloys and their Constituents....8vo 2 50
Text-book of the Materials of Construction....8vo, 5 00
Wood's Treatise on the Resistance of Materials and an Appendix on the Preservation of Timber....8vo, 2 00
Elements of Analytical Mechanics....8vo, 3 00
Wood's Rustless Coatings. (*Shortly.*)

STEAM-ENGINES AND BOILERS.

Carnot's Reflections on the Motive Power of Heat. (Thurston.)....12mo, 1 50
Dawson's "Engineering" and Electric Traction Pocket-book..16mo, mor., 5 00
Ford's Boiler Making for Boiler Makers....18mo, 1 00
Goss's Locomotive Sparks....8vo, 2 00
Hemenway's Indicator Practice and Steam-engine Economy....12mo. 2 00
Hutton's Mechanical Engineering of Power Plants....8vo, 5 00
Heat and Heat-engines....8vo, 5 00
Kent's Steam-boiler Economy....8vo, 4 00
Knight's Practice and Theory of the Injector....8vo 1 50
MacCord's Slide-valves....8vo, 2 00
Meyer's Modern Locomotive Construction....4to. 10 00

Peabody's Manual of the Steam-engine Indicator 12mo, 1 50
Tables of the Properties of Saturated Steam and Other Vapors 8vo, 1 00
Thermodynamics of the Steam-engine and Other Heat-engines 8vo, 5 00
Valve-gears for Steam-engines 8vo, 2 50
Peabody and Miller's Steam-boilers 8vo, 4 00
Pray's Twenty Years with the Indicator ..... Large 8vo, 2 50
Pupin's Thermodynamics of Reversible Cycles in Gases and Saturated Vapors. (Osterberg.) 12mo, 1 25
Reagan's Locomotives: Simple, Compound, and Electric 12mo, 2 50
Rontgen's Principles of Thermodynamics. (Du Bois.) 8vo, 5 00
Sinclair's Locomotive Engine Running and Management 12mo, 2 00
Smart's Handbook of Engineering Laboratory Practice 12mo, 2 50
Snow's Steam-boiler Practice 8vo, 3 00
Spangler's Valve-gears 8vo, 2 50
Notes on Thermodynamics 12mo, 1 00
Spangler, Greene, and Marshall's Elements of Steam-engineering 8vo, 3 00
Thurston's Handy Tables 8vo, 1 50
Manual of the Steam-engine ..... 2 vols. 8vo, 10 00
Part I.—History, Structure, and Theory 8vo, 6 00
Part II.—Design, Construction, and Operation 8vo, 6 00
Handbook of Engine and Boiler Trials, and the Use of the Indicator and the Prony Brake 8vo, 5 00
Stationary Steam-engines 8vo, 2 50
Steam-boiler Explosions in Theory and in Practice 12mo 1 50
Manual of Steam-boilers, Their Designs, Construction, and Operation. 8vo, 5 00
Weisbach's Heat, Steam, and Steam-engines. (Du Bois.) 8vo, 5 00
Whitham's Steam-engine Design 8vo, 5 00
Wilson's Treatise on Steam-boilers. (Flather.) 16mo, 2 50
Wood's Thermodynamics, Heat Motors, and Refrigerating Machines 8vo, 4 00

MECHANICS AND MACHINERY.

Barr's Kinematics of Machinery 8vo, 2 50
Bovey's Strength of Materials and Theory of Structures 8vo, 7 50
Chase's The Art of Pattern-making 12mo, 2 50
Chordal.—Extracts from Letters 12mo, 2 00
Church's Mechanics of Engineering 8vo, 6 00
Notes and Examples in Mechanics 8vo, 2 00
Compton's First Lessons in Metal-working 12mo, 1 50
Compton and De Groodt's The Speed Lathe 12mo, 1 50
Cromwell's Treatise on Toothed Gearing 12mo, 1 50
Treatise on Belts and Pulleys 12mo, 1 50
Dana's Text-book of Elementary Mechanics for the Use of Colleges and Schools 12mo, 1 50
Dingey's Machinery Pattern Making 12mo, 2 00
Dredge's Record of the Transportation Exhibits Building of the World's Columbian Exposition of 1893 ..... 4to, half morocco, 5 00
Du Bois's Elementary Principles of Mechanics:
Vol. I.—Kinematics 8vo, 3 50
Vol. II.—Statics 8vo, 4 00
Vol. III.—Kinetics 8vo, 3 50
Mechanics of Engineering. Vol. I ..... Small 4to, 7 50
Vol. II ..... Small 4to, 10 00
Durley's Kinematics of Machines 8vo, 4 00
Fitzgerald's Boston Machinist 16mo, 1 00
Flather's Dynamometers, and the Measurement of Power 12mo, 3 00
Rope Driving 12mo, 2 00
Goss's Locomotive Sparks 8vo, 2 00

Hall's Car Lubrication.....12mo, 1 00
Holly's Art of Saw Filing.....18mo 75
* Johnson's Theoretical Mechanics.....12mo, 3 00
Statics by Graphic and Algebraic Methods.....8vo, 2 00
Jones's Machine Design:
Part I.—Kinematics of Machinery.....8vo, 1 50
Part II.—Form, Strength, and Proportions of Parts.....8vo, 3 00
Kerr's Power and Power Transmission.....8vo, 2 00
Lanza's Applied Mechanics.....8vo, 7 50
MacCord's Kinematics; or, Practical Mechanism.....8vo, 5 00
Velocity Diagrams.....8vo, 1 50
Maurer's Technical Mechanics.....8vo, 4 00
Merriman's Text-book on the Mechanics of Materials.....8vo, 4 00
* Michie's Elements of Analytical Mechanics.....8vo 4 00
Reagan's Locomotives: Simple, Compound, and Electric.....12mo, 2 50
Reid's Course in Mechanical Drawing.....8vo, 2 00
Text-book of Mechanical Drawing and Elementary Machine Design..8vo, 3 00
Richards's Compressed Air.....12mo, 1 50
Robinson's Principles of Mechanism.....8vo, 3 00
Ryan, Norris, and Hoxie's Electrical Machinery.....8vo, 2 50
Sinclair's Locomotive-engine Running and Management.....12mo, 2 00
Smith's Press-working of Metals.....8vo, 3 00
Materials of Machines.....12mo, 1 00
Spangler, Greene, and Marshall's Elements of Steam-engineering.....8vo, 3 00
Thurston's Treatise on Friction and Lost Work in Machinery and Mill Work.....8vo, 3 00
Animal as a Machine and Prime Motor, and the Laws of Energetics.12mo, 1 00
Warren's Elements of Machine Construction and Drawing.....8vo, 7 50
Weisbach's Kinematics and the Power of Transmission. (Herrmann—Klein.).....8vo, 5 00
Machinery of Transmission and Governors. (Herrmann—Klein.).8vo, 5 00
Wood's Elements of Analytical Mechanics.....8vo, 3 00
Principles of Elementary Mechanics.....12mo, 1 25
Turbines.....8vo, 2 50
The World's Columbian Exposition of 1893.....4to, 1 00

METALLURGY.

Egleston's Metallurgy of Silver, Gold, and Mercury:
Vol. I.—Silver.....8vo, 7 50
Vol. II.—Gold and Mercury.....8vo, 7 50
** Iles's Lead-smelting. (Postage 9 cents additional.).....12mo, 2 50
Keep's Cast Iron.....8vo, 2 50
Kunhardt's Practice of Ore Dressing in Europe.....8vo, 1 50
Le Chatelier's High-temperature Measurements. (Boudouard—Burgess.).12mo, 3 00
Metcalf's Steel. A Manual for Steel-users.....12mo, 2 00
Smith's Materials of Machines.....12mo, 1 00
Thurston's Materials of Engineering. In Three Parts.....8vo, 8 00
Part II.—Iron and Steel.....8vo, 3 50
Part III.—A Treatise on Brasses, Bronzes, and Other Alloys and their Constituents.....8vo, 2 50
Ulke's Modern Electrolytic Copper Refining.....8vo, 3 00

MINERALOGY.

Barringer's Description of Minerals of Commercial Value. Oblong, morocco, 2 50
Boyd's Resources of Southwest Virginia.....8vo, 3 00
Map of Southwest Virginia.....Pocket-book form, 2 00

Brush's Manual of Determinative Mineralogy. (Penfield.).....8vo, 4 00
Chester's Catalogue of Minerals.....8vo, paper, 1 00
Cloth, 1 25
Dictionary of the Names of Minerals.....8vo, 3 50
Dana's System of Mineralogy..... Large 8vo, half leather, 12 50
First Appendix to Dana's New "System of Mineralogy.".....Large 8vo, 1 00
Text-book of Mineralogy.....8vo, 4 00
Minerals and How to Study Them.....12mo, 1 50
Catalogue of American Localities of Minerals.....Large 8vo, 1 00
Manual of Mineralogy and Petrography.....12mo, 2 00
Eakle's Mineral Tables. (*Shortly.*)
Egleston's Catalogue of Minerals and Synonyms.....8vo, 2 50
Hussak's The Determination of Rock-forming Minerals. (Smith.) Small 8vo, 2 00
Merrill's Non-Metallic Minerals. (*Shortly.*)
* Penfield's Notes on Determinative Mineralogy and Record of Mineral Tests.
8vo, paper, 0 50
Rosenbusch's Microscopical Physiography of the Rock-making Minerals.
(Iddings.).....8vo, 5 00
* Tillman's Text-book of Important Minerals and Docks..... 8vo, 2 00
Williams's Manual of Lithology.....8vo, 3 00

MINING.

Beard's Ventilation of Mines.....12mo, 2 50
Boyd's Resources of Southwest Virginia.....8vo, 3 00
Map of Southwest Virginia.....Pocket-book form, 2 00
* Drinker's Tunneling, Explosive Compounds, and Rock Drills.
4to, half morocco, 25 00
Eissler's Modern High Explosives.....8vo, 4 00
Fowler's Sewage Works Analyses.....12mo, 2 00
Goodyear's Coal-mines of the Western Coast of the United States.....12mo, 2 50
Ihlseng's Manual of Mining.....8vo, 4 00
** Iles's Lead-smelting. (Postage 9c. additional.).....12mo, 2 50
Kunhardt's Practice of Ore Dressing in Europe.....8vo, 1 50
O'Driscoll's Notes on the Treatment of Gold Ores.....8vo, 2 00
* Walke's Lectures on Explosives.....8vo, 4 00
Wilson's Cyanide Processes.....12mo, 1 50
Chlorination Process.....12mo, 1 50
Hydraulic and Placer Mining.....12mo, 2 00
Treatise on Practical and Theoretical Mine Ventilation.....12mo 1 25

SANITARY SCIENCE.

Copeland's Manual of Bacteriology. (*In preparation.*)
Folwell's Sewerage. (Designing, Construction and Maintenance.).....8vo, 3 00
Water-supply Engineering.....8vo, 4 00
Fuertes's Water and Public Health.....12mo, 1 50
Water-filtration Works.....12mo, 2 50
Gerhard's Guide to Sanitary House-inspection.....16mo, 1 00
Goodrich's Economical Disposal of Town's Refuse.....Demy 8vo, 3 50
Hazen's Filtration of Public Water-supplies.....8vo, 3 00
Kiersted's Sewage Disposal.....12mo, 1 25
Leach's The Inspection and Analysis of Food with Special Reference to State
Control. (*In preparation.*)
Mason's Water-supply. (Considered Principally from a Sanitary Standpoint.) 3d Edition, Rewritten.....8vo, 4 00
Examination of Water. (Chemical and Bacteriological.).....12mo, 1 25

Merriman's Elements of Sanitary Engineering.....8vo, 2 00
Nichols's Water-supply. (Considered Mainly from a Chemical and Sanitary Standpoint.) (1883.).....8vo, 2 50
Ogden's Sewer Design.....12mo, 2 00
* Price's Handbook on Sanitation.....12mo, 1 50
Richards's Cost of Food. A Study in Dietaries.....12mo, 1 00
Cost of Living as Modified by Sanitary Science.....12mo, 1 00
Richards and Woodman's Air, Water, and Food from a Sanitary Standpoint.....8vo, 2 00
* Richards and Williams's The Dietary Computer.....8vo, 1 50
Rideal's Sewage and Bacterial Purification of Sewage.....8vo, 3 50
Turneaure and Russell's Public Water-supplies.....8vo, 5 00
Whipple's Microscopy of Drinking-water.....8vo, 3 50
Woodhull's Notes and Military Hygiene.....16mo, 1 50

MISCELLANEOUS.

Barker's Deep-sea Soundings.....8vo, 2 00
Emmons's Geological Guide-book of the Rocky Mountain Excursion of the International Congress of Geologists.....Large 8vo, 1 50
Ferrel's Popular Treatise on the Winds.....8vo, 4 00
Haines's American Railway Management.....12mo, 2 50
Mott's Composition, Digestibility, and Nutritive Value of Food. Mounted chart. 1 25
Fallacy of the Present Theory of Sound.....16mo, 1 00
Ricketts's History of Rensselaer Polytechnic Institute, 1824–1894. Small 8vo, 3 00
Rotherham's Emphasized New Testament.....Large 8vo, 2 00
Steel's Treatise on the Diseases of the Dog.....8vo, 3 50
Totten's Important Question in Metrology.....8vo, 2 50
The World's Columbian Exposition of 1893.....4to, 1 00
Worcester and Atkinson. Small Hospitals, Establishment and Maintenance, and Suggestions for Hospital Architecture, with Plans for a Small Hospital.....12mo, 1 25

HEBREW AND CHALDEE TEXT-BOOKS.

Green's Grammar of the Hebrew Language.....8vo, 3 00
Elementary Hebrew Grammar.....12mo, 1 25
Hebrew Chrestomathy.....8vo, 2 00
Gesenius's Hebrew and Chaldee Lexicon to the Old Testament Scriptures. (Tregelles.).....Small 4to, half morocco, 5 00
Letteris's Hebrew Bible.....8vo, 2 25